EFFECT OF TEMPERING MOISTURE AND INFRARED HEATING TEMPERATURE ON THE FUNCTIONAL AND NUTRITIONAL PROPERTIES OF DESI CHICKPEA AND HULL-LESS BARLEY FLOURS, AND THEIR BLENDS

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By
Tian Bai
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ABSTRACT

The overall goal of this research was to investigate the effect of tempering moisture and infrared heating surface temperature on the functional and nutritional properties of Desi chickpea and hull-less barley flours, and their blends. Specifically, chickpea (initial moisture content 6.29%) and barley (initial moisture content 6.65%) seeds were tempered to 20% moisture content or left un-tempered followed by infrared heating to reach a surface temperature of 115 or 135^{o}C. The infrared heating process was conducted independently for three times under the same condition to obtain triplicate samples. The seeds were then milled into flour for the subsequent analysis of their physicochemical and functional properties, levels of anti-nutritional compounds and *in vitro* protein digestibility.

In the first study, the impact of infrared heating surface temperature and tempering moisture on the functional properties of Desi chickpea, hull-less barley, and their blends were examined. Neither of the factors was found to significantly affect the proximate composition (i.e., protein, lipid, and ash) of the flours ($p>0.05$). The content of protein, lipid, and ash was ~25%, 6% and 3% in chickpea flour, and 11%, 2% and 2% in barley flour, respectively. However, the levels of gelatinized starch were found to significantly increase with the combined tempering-heat treatment in each flour ($p<0.05$). The solubility of chickpea and barley proteins were found to be significantly reduced by the combined tempering-heat treatment from 70% and 27% in the untreated samples to 44% and 11% in the treated chickpea and barley flour, respectively due to protein denaturation ($p<0.05$), despite the surface charge of both flours increasing. The oil holding capacity was found not to be affected by either factor ($p>0.05$), which was around 1.1 and 1.3 g/g of chickpea and barley flour respectively, whereas the water hydration capacity was significantly increased from 1.1 and 1.4 to 1.8 and 2.8 g/g of flour in chickpeas and barley respectively ($p<0.05$). The effect of infrared heating and tempering on the emulsion and foaming properties differed between flours. In the case of chickpea flour, emulsion activity (EA) increased and foaming capacity (FC) decreased significantly ($p<0.05$) with the combined tempering-heat treatments, whereas emulsion (ES) and foaming (FS) stabilities showed no significant difference before and

after treatments. In the case of barley flour, EA and ES were found both to decrease with the combined tempering-heat treatment, whereas solutions became non-foaming with either temperature or the tempering-heat combination relative to the control. Based on rapid visco-analysis, the viscosity of the barley and chickpea flour suspensions, in general, had reduced viscosity and increased pasting temperatures both with temperature or the tempering-heat combination. These trends were more pronounced with tempering and at the higher temperature (135^{o}C) in both chickpea ($p<0.05$) and barley flour ($p>0.05$). Based on the aforementioned results, chickpea and barley flours tempered to 20% moisture and heated to 135^{o}C were subsequently blended at chickpea: barley ratio of 20:80, 40:60, 60:40 and 80:20. The physicochemical and functional properties of the blends showed a gradient change in accordance with their blending ratios.

In the second study, the impact of infrared heating surface temperature and tempering moisture on the levels of anti-nutritional factors (i.e., trypsin/chymotrypsin inhibitors, total phenolics and condensed tannins), amino acid composition and *in vitro* protein digestibility properties of Desi chickpea, hull-less barley, and their blends were examined. Results indicated that both temperature and the tempering/temperature treatment caused a reduction in levels of all anti-nutritional factors for both flours, and the effect was more prominent in the tempering-heat combination ($p<0.05$). The amino acid composition of both flours was found not to be substantially changed with tempering or infrared heating. The amino acid scores (AAS) of chickpea and barley flours, as determined by the first limiting amino acid using the FAO/WHO reference pattern found in the case of barley to be limiting in lysine with an AAS of ~0.7, whereas for chickpea flour, threonine was limiting and had an AAS of ~0.9. The *in vitro* protein digestibility of chickpea samples was found to increase from 76% to 79% with the tempering-heat (135^{o}C) combination, whereas barley flour increased from 72% to 79% when directly heated to 135^{o}C (without tempering). *In vitro* protein digestibility corrected amino acid score (IV-PDCAAS) was found to increase from 0.65 to 0.71 for chickpea flour and 0.44 to 0.52 for barley flour, respectively with tempering-heat (135^{o}C) combination indicating that tempering with infrared heating can improve the nutritional value of both flours. The addition of chickpea flour to the barley flour acted to improve the nutritional properties (IV-PDCAAS), to an extent depending on the concentration of chickpea flour present.

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LIST OF SYMBOLS AND ABBREVIATIONS

a*	Redness
AA	Amino acid(s)
AACC	American Association of Cereal Chemists
AAS	Amino acid score
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
b*	Yellowness
BV	Biological value
CDC	Crop Development Centre
CIA	Chymotrypsin inhibitor activity
CFIA	Canadian Food Inspection Agency
d.b.	Dry basis
DIAAS	Digestible Indispensable Amino Acid Score
EA	Emulsion activity
EDTA	Ethylenediaminetetraacetic acid
ES	Emulsion stability
f (kα)	Smoluchowski approximation
FAO	Food and Agriculture Organization of the United Nations
FC	Foaming capacity
FS	Foaming stability
g	Gravitational Force
GS	Gelatinized starch
HMW	High molecular weight
IVPD	*In vitro* protein digestibility

IVPDCAAS	In vitro protein digestibility corrected amino acid score
kDa	Kilodalton
L*	Lightness
NB	Nitrogen balance
NGO	Non-governmental organization
NPR	Net protein ratio
NPU	Net protein utilization
OHC	Oil holding capacity
PER	Protein efficiency ratio
pI	Isoelectric point
PR	Protein rating
SAA	Sulphur amino acid(s)
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
TIA	Trypsin inhibitor activity
TS	Total starch
U_E	electrophoretic mobility
WHC	Water hydration capacity
WHO	World Health Organization
ZP	Zeta potential
κ	Debye length

1. INTRODUCTION

1.1. Overview

Cereals and pulses have been widely consumed as staple foods for centuries around the world. Cereals are classified as grasses and members of the monocot family *Poaceae* which includes wheat (soft and durum wheat), maize (yellow and white maize), millet, oats, sorghum, rice, barley and other minor grains (Pomeranz, 1987). Cereal crops are rich in proteins, carbohydrates, vitamins, and minerals; have low content of fat (Huang et al., 1997); and contain all essential amino acids (AA) needed to support growth other than lysine which is considered limiting. In contrast, pulses are classified as a grain legume, grown for their edible seeds contained within the pod of a leguminous crop. Pulses include dried peas, lentils, chickpeas and dried beans such as faba, kidney, navy and pinto beans. They have the advantage of being able to fix nitrogen and as such are often used in crop rotation under normal agronomic practices. Similar to cereals, pulses are rich in proteins and carbohydrates; are low in fat with the exception of chickpeas; and contain all the essential AA with the exception of cysteine and methionine which are limiting. Pulses are also high in dietary fibre, B-complex vitamins, folic acid, potassium, calcium and iron (Bellido et al., 2003). Based on their limiting essential AA, cereals and pulses are often recommended to be consumed together to ensure the individual receives all the necessary amino acids to support growth and maintenance from their diet.

Cereals and pulses also contain anti-nutritional compounds at varying concentrations depending on the compounds and species, that can negatively impact protein digestion (e.g., trypsin and chymotrypsin inhibitors, phenolic compounds and tannins), starch digestion (e.g., α-amylase inhibitor), mineral absorption (e.g., phytates and oxalates) or intestine function (e.g., lectins). However, these can be significantly reduced or eliminated through processing by physical means (e.g., dehulling and air classification) (Tiwari and Singh, 2012), fermentation (Khattab and Arntfield, 2009), germination (Vidal-Valverde et al., 1994) or other unit operations, such as canning (Pedrosa et al., 2015), extrusion (Alonso et al., 2000; Frias et al., 2011), roasting (Sharma et al., 2011), microwave cooking and infrared heating (Deepa and Hebbar, 2016) to improve their

nutritional value. In this research, the impact of infrared heating under varying conditions will be investigated as a means of improving the protein quality and functionality of chickpea and barley flours and their blends.

Barley is one of the most important staple food in sub-Saharan Africa with high yield. Barley contains about 60 to 64% carbohydrates, 8 to 15% protein, 2 to 3% lipids and 2 to 3% minerals (MacGregor and Bhatty, 1993). It has been proved to be a health-promoting cereal and is now a trending ingredient in many processed foods such as breakfast cereals, snack food and bread (Altan, 2014). Barley is high in β-glucan (3.6 to 6.1%) which is good for the intestinal health and is effective in lowering blood cholesterol and controlling the glycemic index (Sharma et al., 2011). Barley is mostly utilized as feed and the rest mainly for malting which account for around two thirds and one third of the production separately (Baik and Ullrich, 2008). Regarding the food application of barley, barley is prevalent in African countries especially in Ethiopia where barley is considered as the No. 1 food crop and used as an ingredient in beverages and snacks (Abraha et al., 2013; Mohammed et al., 2016). The utilization of barley is limited by its non-digestible hull which can have a negative impact on the digestibility as well as the time-consuming and profit-draining dehulling process (Thacker, 1999). As a result, the hull-less type of barley is considered to have much greater potential. Studies also have shown that hull-less barley has a higher content of β-glucan, protein, and better flour yield compared to other types of barley (Zhang et al., 2002; Soares et al., 2007; Bhatty, 1997).

Chickpeas became the second most produced pulse crops second only to beans, surpassing peas in 2009 (FAO, 2016) and Canada and Ethiopia are both major countries that produce chickpeas. In Canada, Saskatchewan accounts for around 80% of its production of chickpea. Chickpeas are high in protein (17% to 31%), carbohydrates (60% to 67%), lipids (2.9% to 6.9%) and minerals (2.3% to 3.8%), with relatively low levels of anti-nutritional factors (Asif et al., 2013; Jukanti et al., 2012; Wang and Daun, 2004). It has also been shown to have nutritional benefits such as its use for controlling weight, diabetes and cholesterol (Roy et al., 2010). Chickpeas can be classified into two varieties: Desi and Kabuli. Desi chickpeas are smaller in size, darker in appearance and have a thicker seed coat, whereas Kabuli chickpeas are larger in size and have thinner seed coat (Boye et al., 2010). Desi chickpeas account for 80% of the world chickpea production (Bard, 2016), whereas Kabuli chickpeas are more dominant in Canada. Desi chickpeas tend to have shorter maturation time (110 days) and require lower seeding temperature (7^{o}C)

compared to Kabuli chickpeas (110-120 days to maturity, 10ºC of seeding temperature) (Saskatchewan Pulse Growers, 2017).

The current research uses hull-less barley and Desi chickpeas as the selected materials to show the impact of tempering moisture and infrared heating surface temperatures on the physicochemical and nutritional properties of their flours and blends. Infrared heating involves the application of infrared radiation to the surface of the seed. The radiation penetrates the surface to induce vibrational changes and the rotation of water molecules to generate heat (Emami et al., 2010a). Often in the case of cereal and pulses, tempering is used prior to processing to increase the moisture within the seeds, and thus the degree of vibrational energy is enhanced. Depending on the tempering moisture and the surface temperature generated by infrared heating, various changes to the protein quality (i.e., digestibility) and functionality in the resulting flours, and the levels of anti-nutritional factors can occur (Melcion and Valdebouze, 1977; Arntfield et al., 2001; Zarkadas and Wiseman, 2002). The knowledge gained as part of this study, particularly as it pertains to improving protein quality of the flours, will be used in the development of therapeutic food aid products (work not included in this research) targeting individuals in Ethiopia suffering from moderately acute malnutrition. Barley and chickpeas are widely grown in Ethiopia, whereupon adoption of the developed products by NGOs or the United Nation's World Health Organization/Food and Agriculture Organization, ingredients could be sourced locally to support economic growth in the region. In addition, infrared heating technology is available in Africa for processing. Ethiopia makes an ideal case study for this work because of the large food insecurity issues and cases of malnutrition being faced within the country.

1.2. Objectives

The overall goal of this research is to investigate the impact of tempering moisture and seed surface temperature generated by infrared heating on the functional and nutritional properties of chickpea and barley flours, and their blends. Specific objectives include the following:

- To test the effect of tempering moisture and surface temperature on the physicochemical and functional properties (i.e., solubility, pasting, foaming and emulsion properties) of Desi chickpea and hull-less barley flours, and their blends.
- To test the effect of tempering moisture and infrared heating temperature on the nutritional

properties (i.e., levels of anti-nutritional factors, amino acid composition and *in vitro* protein digestibility) of Desi chickpea and hull-less barley flours, and their blends.

1.3. Hypotheses

The following hypotheses will be tested during this research:

- As tempering moisture and surface temperature increases, protein functionality will be improved due to partial protein denaturation and starch gelatinization.
- As tempering moisture and surface temperature increases, the levels of anti-nutritional factors will decline.
- As tempering moisture and surface temperature increases, the protein digestibility will be improved due to partial protein denaturation and the decrease of anti-nutritional factors.
- Protein quality will be improved by blending in the chickpea flour to the barley flour, whereas functionality will reflect range in-between both flours.

2. LITERATURE REVIEW

As the cost of animal proteins is on the rise, the food industry is searching for alternative protein sources of high quality in terms of both their nutrition and functionality, with lower production costs and free from allergens. Protein quality is also of significant importance in order to address some of the food security issues being faced around the globe where access to a high-quality protein source is limited by several issues including environmental effects, inadequate irrigation, war, famine, political unrest and so on. The lack of a low-cost and well-balanced protein source that is abundant is important in order to help resolve some of the issues surrounding malnutrition in high-risk regions around the globe (e.g., sub-Saharan Africa, Central America, and Southern Asia). Community health education and food assistance programs (e.g., United Nation's World Food Programme) are promoting the consumption of complementary foods in order to provide a well-balanced diet. In particular, using mixtures of cereals and pulses to provide a high-quality protein source for human nutrition, as each individually lacks 1 to 2 essential AA important to support growth and development.

Plant proteins can be separated into four main classes based on their solubility using the Osborne fractionation procedure (Osborne, 1895). For instance, albumins are considered to be water-soluble, whereas globulins, prolamins, and glutelins are soluble in salt solutions (10% NaCl), alcohol (70-90% ethanol) and weak acids/bases, respectively (Yada, 2004). Proteins can also be classified based on their location within the seed (proteins of the endosperm, aleurone layer, and embryo), their function (storage proteins, protective proteins and structural and metabolic proteins) or chemical composition (simple proteins and complex proteins). Pulses tend to be higher in globulin- and albumin-type proteins, whereas cereals are higher in prolamins. Each provides a different AA profile, conformation and functionality, leading to differences in protein quality. In order to improve the protein quality of both pulses and cereals, research has focused on effects of minimal processing (e.g., germination/sprouting; milling) and thermal processing (e.g., canning, roasting and infrared heating) as a means to altering the protein structure. Depending on the treatment and the food application partial or complete denaturation may ensue leading to different

functionality and digestibility. Blending of cereals and pulses are also of significant importance in order to enhance their nutritional value. Processing also has the added advantage of reducing or eliminating levels of anti-nutritional factors that interfere with protein digestion.

2.1. Pulses

Pulses are the dry edible seeds from the pods of crops from the *Leguminosae* family, which include peas (*Pisum sativum* L.), chickpeas (*Cicer arietinum* L.), lentils (*Lens culinaris* L.), faba beans (*Vicia faba* L.), and dry beans (*Phaseolus vulgaris* L.). They are commonly consumed as food or feed by humans or domestic animals as dried seeds and used to be considered as "the meat of the poor" because of their comparable nutritional value at low price. Pulses represent an excellent source of protein (21-25%), carbohydrates (60-65%), dietary fibre (e.g., cellulose, hemicellulose, polysaccharides, and lignin), vitamins, minerals (e.g., potassium, folate, calcium and phosphorous) as well as some phytochemicals (Tiwari and Singh, 2012). With the exception of chickpeas (3-7%), pulses also tend to be low in fat (~1%) (Jukanti et al., 2012). The proteins are highly nutritious as they are high in lysine and contain all of the essential AA with the exception of the sulphur-containing AA, methionine and cysteine (Deshpande et al., 1982; Vaz Patto et al., 2015). As such, they are commonly consumed with cereal grains, which are high in the sulphur-containing AA but low in lysine (Vaz Patto et al., 2015).

Pulse proteins are dominated by both globulin (salt-soluble) and albumin (water-soluble) proteins, accounting for 60-80% and 15-25%, respectively of the total proteins (Tiwari and Singh, 2012). The globulin proteins comprise of two major types: legumin (11S, S is the Svedberg Unit; molecular mass ~350-400 kDa) and vicilin (7S; molecular mass ~180 kDa) (Tzitzikas et al., 2006). The legumin protein is a hexamer, comprise of six subunits consisting of an acidic α- (molecular mass ~40 kDa) and basic β- (molecular mass ~ 20 kDa) linked chains held together by disulphide bonds, and devoid of any carbohydrate (Tiwari and Singh, 2012). In contrast, vicilin is a trimer comprised of 3 subunits held together by non-covalent interactions and has glycosylated subunits. A third globulin protein is also known as convicilin (molecular mass ~290 kDa, with subunits of ~71 kDa in size), which is non-glycosylated and has sulphur AA which does not appear in the vicilin structure (Croy et al., 1980). Albumin proteins consist of enzymes, protease inhibitors, lipoxygenase and lectins (El Fiel et al., 2002; Roy et al., 2010).

2.2. Cereals

Cereal crops are considered to be a grass from the family *Poaceae*, grown for their edible endosperm, germ and bran. Cereals include: corn (*Zea mays* L.), rice (*Oryza sativa* L.), wheat (*Triticum* L.), barley (*Hordeum vulgare* L.), sorghum (*Sorghum bicolor* Moench), millet (*Pennisetum typhoides* L.), oats (*Avena sativa*), rye (*Secale cereals* L.) and teff (*Eragrostis tef* Trotter); with the vast majority of production (~90%) being corn, rice and wheat globally (in order from highest to lowest). Cereal proteins are dominated by prolamin- and glutelin-type proteins. Prolamins are the major storage proteins in most cereals with the exception of oats and rice, of which the major storage proteins are 12S globulins and glutelins, respectively (Muench and Okita, 1997). They are rich in leucine, proline and glutamine, however, are deficient in lysine and tryptophan (El Fiel et al., 2002). Prolamins are commonly known as gluten in wheat, as zein in maize, as kafirin in sorghum, as hordein in barley, as secalin in rye, and as avenin in oats (Shewry and Tatham, 1990; Shewry and Halford, 2002). According to Shewry and Tatham (Shewry and Tatham, 1990), prolamins in wheat and its close species (barley and rye) can be divided into three groups: sulphur-rich (S-rich), sulphur-poor (S-poor) and high molecular weight (HMW) prolamins; The S-rich prolamins have the molecular weight of 36 to 44 kDa by SDS-PAGE with proline-rich repeats at N-terminus and cysteine residues at C-terminus, whereas the S-poor prolamins refer to the ω-gliadins in wheat, C-hordeins in barley and ω-secalins in rye with the molecular mass of 36-78 kDa; The HMW prolamins consist of two domain structures (repetitive structures in the center and non-repetitive structures at N- and C- terminus), which are high in glycine, glutamine and proline; They also have two types: an x-type with a molecular mass of 83-88 kDa and a y-type with a molar mass of 67-74 kDa; Glutelins have a lower molecular mass (2.5-21.8 kDa) and are bridged by disulphide bonds.

2.3. Starches in cereals and pulses

Starches in grain crops are the dominant carbohydrates which provide a direct source of energy within the diet (Priestley, 1979; Svihus et al., 2005). Pulses contain 22 to 45% of starch of their total seed weight (Hoover and Sosulski, 1991) and barley is reported to contain 58 to 67% of starch (Chang and Lv, 2017). Starches are widely added in liquid or semi-liquid food products (e.g. soups and sauces) to increase their mouthfeel, consistency and thickness (Ramaswamy et al., 1995). Therefore, the properties of starch will affect the application of flour. Starch consists of two types

of polysaccharides: amylose and amylopectin. The former comprises of linear polymers of glucopyranose units linked together via α-D-(1→4) glycosidic linkages, and has a molecular mass ranging between 10^5 and 10^6 kDa, whereas amylopectin, which is predominant (~70%) in most wild-type species, is highly branched with glucopyranose units linked together by both α-D-(1→4) and α-D-(1→6) glycosidic linkages, and has a molecular mass ranging from 10^7 to 10^9 kDa (Donald, 2004; Tiwari and Singh, 2012). Amylose and amylopectin both reside within the starch granule and have an amorphous and semi-crystalline structure, respectively (Svihus et al., 2005). In terms of pasting properties, amylopectin had higher swelling power than amylose after water absorption, leading to a higher viscosity during pasting test (Yu et al., 2015). Based on the result of X-ray diffraction which is often used to examine the crystalline structure, starches can be categorized into an A-type, B-type or C-type pattern (Sajilata et al., 2006; Arce-Arce et al., 2014). A-type patterns, most common in cereals, comprise of densely packed double helices that form a crystalline structure, with little bound water; B-type patterns are less densely packed crystalline regions with more of an open structure and higher levels of hydration (e.g., commonly found in tubers); and C-type patterns, most commonly found in pulses are mixtures of both A and B-types with B-type structure in the centre and the A-type structure in the outward area (Figure 2.1) (Tiwari et al., 2011). The B-type granules could be transformed to A-type by removing the water in the open structure in B-type granules but the A-type could only be transformed to B-type granules by gelatinization (Imberty et al., 1991). B-type granules have been demonstrated to contain less amylose, higher swelling power and higher gelatinization temperature than A-type granules (Yu et al., 2015). Hoover et al. (2010) summarized that legume starch usually shows a C-type X-ray diffraction pattern. Chang and Lv (2017) observed an A-type crystalline pattern in hull-less barley starch.

As starch granules are heated in water, the amorphous amylose regions absorb water and swell (Singh et al., 2007). Water further diffuses into the semi-crystalline regions dominated by the larger amylopectin polymers resulting in some loss in crystallinity and birefringence under polarized light (as evident by the loss of the 'Maltese cross') (Dendy and Dobraszczyk, 2001). As swelling increases, the granule ruptures leading to leaching of the amylose chains into the aqueous solution. This process is known as gelatinization (Cui, 2005). The temperature at which this occurs varies depending on the source of starch, solvent conditions (e.g., pH, types, and concentration of salt) and presence of other macromolecules within the solution (e.g., fat, proteins and sugars) (Cui,

2005; Hirashima et al., 2012). Gelatinization temperatures and enthalpy of gelatinization are determined typically using differential scanning calorimetry (DSC). Chickpeas have been reported to have a gelatinization temperature range of 60 to 78ºC (El Faki et al., 1983) and the gelatinization temperature of barley starch was between 59 to 65ºC (Chang and Lv, 2017). Gelatinized starch has a stronger ability of binding water than native starch. Lv et al. (2011) reported that 97% gelatinized maize starch had a water retention of 22% whereas native starch had only 4.3% at temperatures between 100 to 200ºC.

Upon cooling, the heated starch solution thickens as the starch recrystallization occurs and water is excluded from the starch granules, a process known as retrogradation (Thirathumthavorn and Charoenrein, 2005; Vaz Patto et al., 2015). The process restricts the amount of available amylose for hydrolysis reactions (e.g., during digestion) (Chung et al., 2006) and has an evident effect on food quality (e.g., the staling of bread) (Boye, 2004).

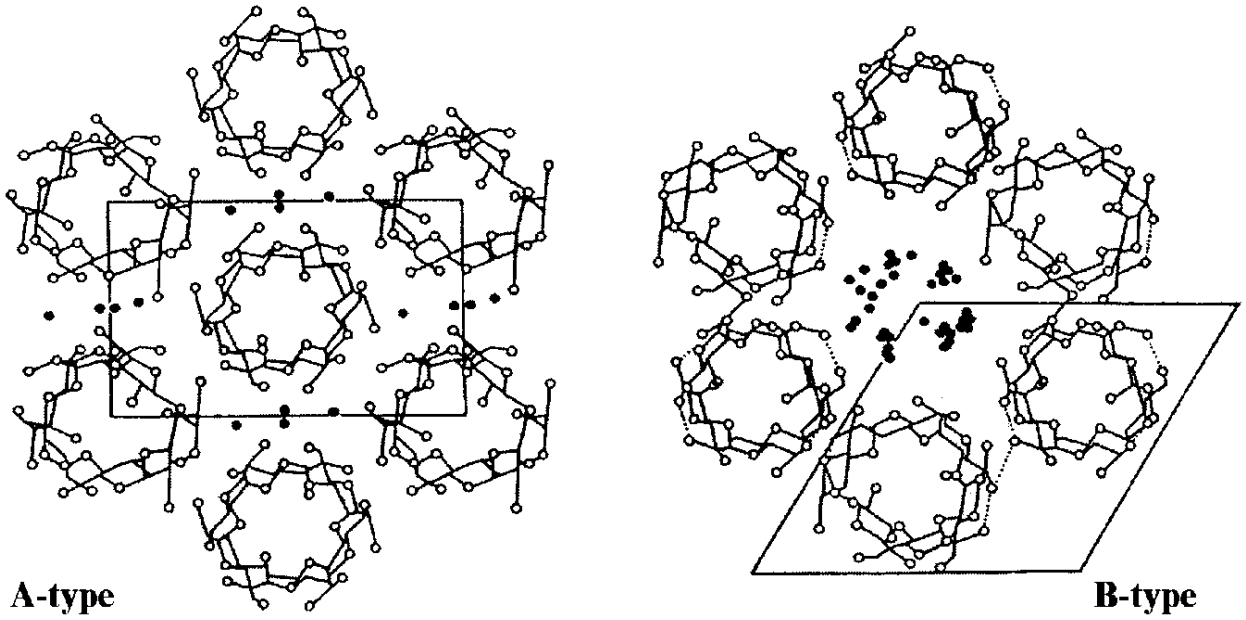

Figure 2.1 The crystal structure of A-type and B-type crystalline starch. The small black circles represent water molecules within the structure; glucose molecules are represented by open circles (Donald, 2004).

2.4. Anti-nutritional factors (ANFs) present in pulses and cereals

Protein digestibility tends to vary within the pulses but is generally lower than that of cereal and animal proteins due to the presence of anti-nutritional factors (Tiwari and Singh, 2012; Rehman et al., 2014). Pulses typically contain much higher levels of these compounds than cereals. The anti-nutritional compounds include:

- *Phenolic compounds*, such as flavonoids, phenolic acids, and tannins, which act to reduce protein digestibility and mineral bioavailability. Phenolic compounds tend to be richer in dark pigmented cultivars.
- *Enzyme inhibitors,* such as chymotrypsin inhibitors, trypsin inhibitors and amylase inhibitors, which act to reduce digestibility by inhibiting enzymes needed to break down both proteins and carbohydrates. These inhibitors are heat labile and can be eliminated by thermal processing.
- *Phytates,* which act to inhibit the bioavailability of minerals such as phosphorus, calcium, iron, and zinc.
- *Lectins,* which have been reported to cause diarrhea, vomiting and red blood cell agglutination.
- *Oxalates,* which interact with minerals, such as Ca^{2+} and Fe^{2+} to reduce their absorption.
- *Saponins,* which act by interacting with bile acid and cholesterol and may lead to an excessively high level of cholesterol.
- *Oligosaccharides,* which can be associated with flatulence as they are fermented by bacteria within the gastrointestinal tract to cause discomfort (Rehman et al., 2014).
- And*, Vicine and convicine,* which are glycosides found only in faba beans that are associated with favism in certain individuals (Rehman et al., 2014).

Among these anti-nutritional factors, phenolics especially tannins, trypsin inhibitor activity and chymotrypsin inhibitor activity have a close relationship with the protein digestibility. Pulse seeds are rich in trypsin and chymotrypsin inhibitors than other protease inhibitors (Srinivasan et al., 2005), which may limit the application of pulse crops. Most of the trypsin and chymotrypsin inhibitors in pulses have also been found in cereals but they exhibited lower activity of inhibition (Mikola and Suolinna, 1969; Mosolov and Valueva, 2005).

Phenolics refers to a group of substances that contain one or more hydroxyl groups binding to an aromatic ring, and include lignans, flavonoids, neoflavonoids and tannins. These compounds

are known for their ability to strongly cross-link proteins to impair digestion (Waterman and Mole, 1994), act as enzyme inhibitors and may cause astringent tastes (Jukanti et al., 2012). Condensed tannins are the most prevalent type of tannins existing in plants, and are comprised of polyhydroxy flavan-3-ol oligomers linked by carbon-carbon bonds, with the ability to form cross-linking with proteins, starches, metal ions and polysaccharides (Amoako and Awika, 2016; Schofield et al., 2001). The interaction between tannins and starches was found to lead to an increase in the amount of slowly digestible starch and resistant starch (Amoako and Awika, 2016), whereas their cross-linking ability with lysine or methionine in proteins may restrain protein digestibility (Vidal-Valverde et al., 1994).

Trypsin and chymotrypsin are endopeptidases that cut proteins at different sites for digestion. Trypsin and chymotrypsin inhibitors in plants are low molecular weight proteins that are used to protect seeds against bacteria and dormancy (Guillamón et al., 2008). However, in the human body trypsin or chymotrypsin inhibitors mimic the protein substrate by binding to lysine, serine and arginine residues of trypsin, and hydrophobic residues (e.g. leucine, phenylalanine, histidine, and tyrosine) of chymotrypsin to reduce their ability of those enzymes to digest proteins (Dantzger et al., 2015). There are two major types of trypsin inhibitors: the Kunitz type and the Bowman-Birk inhibitors. The former inhibitor is comprised of larger polypeptides (~20 kDa) with two disulphide bonds that can only bind to trypsin, whereas Bowman-Birk type inhibitor is comprised of smaller polypeptides (~8 kDa) with seven disulphide bonds and can bind to both trypsin and chymotrypsin (Jukanti et al., 2012).

Although breeding programs over the past decades have reduced levels of some of these anti-nutritional factors, crop health tends to be adversely affected (Khattab and Arntfield, 2009). From a human and animal nutrition perspective, reducing levels of these factors may be more realistic through different pre-treatment conditions (e.g., dehulling, soaking or milling), traditional processing methods (e.g., germination or fermentation) and advanced processing methods (e.g., micronization, extrusion or microwave heating) (Patterson et al., 2017). For instance, Deshpande et al. (1982) reported dehulling lowered tannin levels in nine types of dry beans (*Phaseolus vulgaris* L.) including Sanilac, Great Northern, Small White, Cranberry, Viva Pink, Pinto, Light Red Kidney, Dark Red Kidney and Black Beauty. Quinteros et al. (2003) reported the oxalate content in beans, chickpeas, and lentils to be reduced upon cooking, with the greatest effects occurring upon industrial processes versus home cooking practices.

2.5. Infrared heating (or micronization)

The technique of infrared heating initiated in the 1970s but only gained its significance in recent years (Deepa and Hebbar, 2016). Infrared heating uses electromagnetic radiation to cause water molecules within the seed to vibrate and generate heat (Fasina et al., 2001). Infrared radiation is sub-divided into three categories: near-infrared (750 nm - 3,000 nm), mid-infrared (3,000 - 25,000 nm) and far-infrared (25,000-100,000 nm) based on their wavelength spectra (Sakai and Hanzawa, 1994). Micronization refers to radiation between wavelengths of 1,800 and 3,400 nm (Emami et al., 2010a). Its high efficiency of electric to heat energy conversion, uniformity of heating, ease of operation and low cost has led to an increasing popularity of infrared drying. A schematic of a small-scale infrared heater is shown in Figure 2.2. In brief, seeds are placed onto the feeder through the hopper, and then delivered by vibration until they reach the end of the conveyor. The surface temperature of the seeds is controlled by altering the speed of the conveyor and the exposure time.

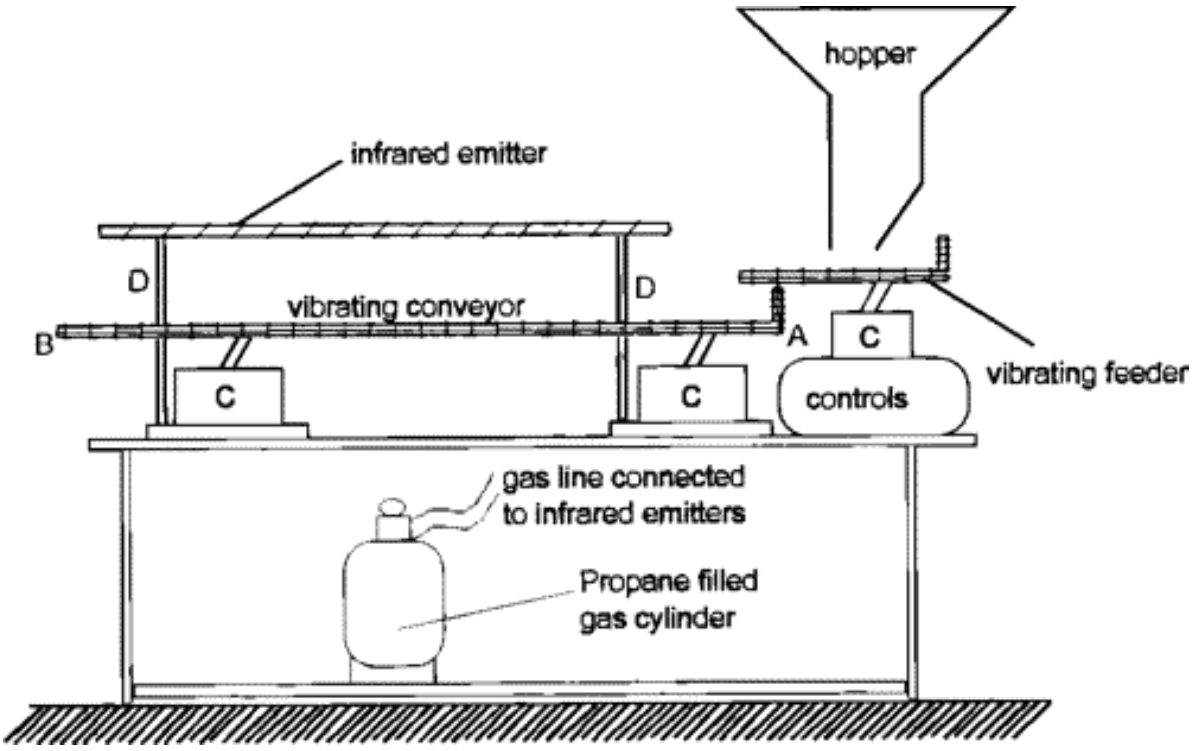

Figure 2.2 A laboratory-scale micronization system with vibrating conveyor (A to B), magnetic vibrator (C) and emitter supporter (D) (Zheng et al., 1998).

Often the drying technique is used to pre-cook cereal and/or pulses in order to reduce cooking times, alter flavour profiles (i.e., reduction of the beany flavour in pulses), reduce the levels of anti-nutritional factors and, improve the functionality and digestibility of the resulting flours (Sun et al., 2006; Mwangwela et al., 2007b; Emami et al., 2010b; Žilić et al., 2010; Kayitesi et al., 2013). Micronization has also been applied in feed processing to improve digestibility and the nutritional quality of animal feed, in cocoa production to help remove the bean skins in order to increase yield, and as a means to reduce microbial counts (Arntfield et al., 1997; Sun et al., 2006).

2.6. Tempering

Tempering is a process that involves soaking the cereal and legume grains in various aqueous solutions, to allow water to be absorbed into the grains until a temperature - moisture equilibrium is established. Since proteins in pulses were bound by non-covalent, hydrogen and hydrophobic bonds, soaking in solutions with different ionic strength, pH or with chelating agents is expected to change the protein structures and interactions. Studies have shown tempering made proteins more susceptible to heat treatments: Arntfield et al. (1997) found lentils tempered to 25% moisture content with water had significantly higher protein solubility than those tempered to 29% and 33%, indicating fewer proteins were denatured when tempered to a lower moisture content (25%) than to a higher moisture content (29% and 33%); Garcia-Vela et al. (1991) reported that pH of the tempering solution did not have much effect on the hardness of black beans and the denaturation temperature of seeds in a carbonate solution (0.1% $NaHCO_3$ and 2.5% K_2CO_3 at pH 11) significantly changed comparing to those in distilled water thus they inferred that soaking in salts may make proteins easier to denature than water. Since starch gelatinization is largely affected by the moisture content within the system, tempering is also expected to have an effect on starch structures as well. In the study by Arntfield et al. (1997) mentioned above, they also reported a negative correlation between maximum force and concentration of gelatinized starch, indicating an increase of gelatinized starch content associated with a softer structure.

Tempering is also an important attribute for milling and dehulling of the seed after the heat treatment. Tempering increases the moisture content within seeds to weaken protein-starch interactions. Without tempering the bran will break into small fragments when milled, making it difficult to separate the bran from the endosperm in cereal grains. Overall, having less fragmented

seeds will lead to improved uniformity of the flour and enhance quality (Hoseney and Delcour, 2010; Alsaffar, 2011).

The popularity and nutritional benefits of pulses are hindered by their long cooking time and presence of anti-nutritional factors. Tempering has been proven to be effective in shortening the cooking time. The addition of water acts to loosen the bonds and interactions within the seeds and to generate pores as water evaporates to increase the rates of hydration. Scanlon et al. (2005) found lentils tempered to 17 to 45% moisture prior to micronization increased the rate of hydration. Similarly, Arntfield et al. (1997) reported that the texture of lentils was softened when tempered to 25 to 35% moisture content followed by infrared heating for 15 min. Increased porosity and higher hydration rates are critical indicators of a reduction in cooking time (Scanlon et al., 2005). There have been many studies on the reduction of cooking time via infrared heating with tempering. Cenkowski and Sosulski (1997) demonstrated that cooking time could be significantly shortened from 30 min to 15 min for lentils (tempered to 26% moisture) when micronized for 55 s at 130ºC. Later, they reported that the cooking time of split peas (tempered to 26% moisture) was reduced by a third when micronized for 90 s with a 500W infrared tubular quartz lamp (seed temperature around 125ºC) (Cenkowski and Sosulski, 1998). Higher tempering moisture showed a more substantial reduction in cooking time. Ndungu et al. (2012) tempered the cowpeas to 25% moisture content and micronized for 5 min at 150ºC and found the cooking time reduced by 14%. In another study on the cooking time of cowpeas, the samples were tempered to 41% prior to infrared heating for 6 min at 153ºC. In this study, cooking time was reduced by 28-49% (Kayitesi et al., 2013).

Tempering is also found to be an effective pre-treatment of infrared heating to reduce the levels of anti-nutritional factors. Yalcin and Basman (2015) examined levels of the trypsin inhibitor activity and lipoxygenase activity in soaked (30 min or 45 min) and un-soaked soybeans with infrared heating and found the soaked soybeans had much lower activities than the un-soaked ones, concluding that soaking is an effective pre-treatment to reduce levels of anti-nutritional factors. In the study on yellow field peas (*Pisum sativum*) (Wang et al, 2003), trypsin inhibitor activity was reduced by 4.5 to 8.8%, and the oligosaccharides including raffinose, stachyose and verbascose were reduced around 8%, 12% and 10%, respectively after tempering. For soybeans, the trypsin inhibitor content was reduced to 87%, 66% and 40% after being micronized at 125ºC, 140ºC and 150ºC, respectively (Žilić et al., 2012). Žilić et al. (2013) also reported the total phenolics were significantly reduced in maize tempered to 11% moisture after infrared heating for 35-60 s at 110ºC.

Levels of these factors further declined as the surface temperature increased from 110ºC to 140ºC. Although some studies found tempering alone can decrease the levels of anti-nutritional factors, accompanying with infrared heating showed a more substantial decline in tannins and trypsin inhibitor activity (Khattab and Arntfield, 2009).

Bellido et al. (2006) compared the effect of tempering using different soaking solutions, including: deionized water, ethylenediaminetetraacetic acid (EDTA, pH 5.3), a citric and ascorbic acid mixture (pH 2.2), and a sodium carbonate, bicarbonate and dibasic sodium phosphate mixture (pH 9.8). The navy bean and black bean seeds were tempered to ~ 27 g/100 g of moisture and then heated to 112-117ºC and their texture, colour, soluble protein and gelatinization properties were evaluated. Seeds tempered with salt solution and water showed superior texture with higher gelatinized starch content, lower soluble protein content, hardness and firmness which are all indicators of a shorter cooking time. But the difference between the results from water-tempered and salt tempered seeds were not significantly different. Therefore, they concluded that tempering with water is sufficient as a tempering solution before infrared heating. Although the salt solution was found to have an equivalent effect in reducing cooking time comparing to water, Scanlon et al. (1998) reported that tempering with salt solution left coloured deposits on the seeds and caused undesired taste. Previous studies also reported that when seeds were tempered to the moisture content lower than 20%, water molecules played the most important role in reducing cooking time, and only when the moisture content approached 40%, the presence of salts took effect (Arntfield et al., 1997; Scanlon et al., 1998).

2.7. Effect of infrared heating and tempering on proteins and starches

The impact of infrared heating on proteins is more substantial than starches, where depending on the surface temperature and tempering moisture, partial or complete protein denaturation may occur. For instance, Fasina et al. (1999) reported pearled barley un-tempered (12.2% moisture) and heated to 135ºC resulted in protein denaturation, whereas at moisture levels >19.3% and temperatures >115ºC denaturation was not found. Protein denaturation is caused by the disruption of hydrogen bonding and other non-covalent interactions at higher temperatures which causes the quaternary and tertiary structures of the proteins to disassociate or unfold to expose buried hydrophobic and hydrophilic moieties to the surface (Sikorski, 2001). Denaturation of proteins can be monitored using differential scanning calorimetry (Fitzsimons et

al., 2007), spectrofluorometry (Hayakawa et al., 1996), circular dichroism spectroscopy or indirectly by solubility, which declines rapidly once the protein is denatured. The change in surface characteristics and protein conformation at the denaturation temperature can have a substantial impact on functionality.

Denaturation tends to cause a decrease in protein solubility, which is an important prerequisite to other functional properties, such as emulsification, foaming, and gelation. Protein solubility is related to the balance between protein-protein and protein-solvent interactions, where the latter promotes greater hydration and solubility (Schwenke, 2001). This balance is highly dependent upon extrinsic factors such as: pH, temperature, ionic strength and protein concentration (Dissanayake et al., 2013; Renkema et al., 2002) and intrinsic factors, such as: protein structure, molecular mass, surface charge and hydrophobicity (Kramer et al., 2012; Tiwari and Singh, 2012). Zheng et al. (1998) reported reductions in protein solubility in water from 37.6% to 32.6% for rye, from 24.7% to 18.5% for triticale, from 18.4% to 14.9% for wheat, from 16.8% to 7.8% for millet and, 4.4% to 3.5% for wild rice when micronized to a surface temperature of 115ºC without tempering. The same authors reported a reduction in solubility from 24.2% to 14.2% for green peas, from 25.9% to 17.9% for yellow peas, from 26.9% to 23.7% for kidney beans, from 24.9% to 15.8% for black beans, from 23.7% to 16.3% for lentils and from 24.7% to 17.4% for pinto beans when micronized to a surface temperature of 140ºC. Arntfield et al. (2001) reported a reduction in protein solubility from ~75% to 50% in lentils tempered to 33% moisture when micronized at 138ºC. A similar decrease in protein solubility was also reported in hull-less and pearled barley (tempered from 13% to 19% and 26% moisture and processed at 115, 135 and 150ºC), red and white spring wheat (tempered to 16 and 22% moisture; 100 ± 5ºC), and sorghum (processed at 102, 250 and 282ºC without tempering) (Fasina et al., 1999; Shiau and Yang, 1982; Sun et al., 2006) with increasing micronization temperatures.

Water holding or absorption capacity (WHC) of proteins refers to the amount of water (by weight) a gram of protein can hold or absorb (Boye et al., 2012). It is an important indicator of bakery applications since higher moisture content in the dough improves the handling characteristics and help retain freshness in bakery products (Ma et al., 2011). WHC relates to protein-water and water-water interactions, where partial protein denaturation has been shown to improve WHC due to an increased exposure of additional hydrophilic moieties and a more open protein structure to entrap or abide water (Arce-Arce et al., 2014; Tiwari and Singh, 2012).

Mwangwela et al. (2007b) found that the WHC of cowpea flour was significantly increased after the seeds (tempered to 41% moisture) were micronized at 130°C for 3 min. Similarly, Fasina et al. (1999) found that the WHC for flour from hull-less pearled barley tempered to 26% moisture and micronized to a surface temperature of 115°C increased when the surface temperature was raised to 135°C, a phenomenon attributed to protein denaturation.

Oil holding capacity (OHC) is the amount of oil (gram) that can be absorbed by one gram of protein closely related to the palatability of food and the retention of the flavour (Tiwari and Singh, 2012). Similar to WHC, protein denaturation enables buried hydrophobic moieties to become exposed and the protein conformation to be more open. As a result, proteins can absorb more oil through hydrophobic interactions. Ma et al. (2011) found the OHC of green lentils increased by 70.7% whereas in Desi chickpeas it increased by 22.5% after boiling. Abbey and Ibeh (1988) reported that cowpea flour after autoclaving increased from 2.9 to 3.2g/g.

Infrared heating causes temperature increase and with the presence of water, starch granules will gelatinize and hence the properties of starch may change. In the study on infrared heated cowpea flour, the overall pasting curve of the cowpea flour heated at 170°C was significantly lower than the flour without infrared heating and no peak was found in the 170°C-heated flour (Mwangwela et al., 2007a). Deepa and Hebbar (2014) found the content of rapidly digestible starch in maize flour had a 7% increase after infrared heating at 159 and 166°C with 40% moisture content. In animal tests, corn and sorghum treated by infrared were found to have higher *in vitro* starch digestibility than the untreated samples (Douglas et al., 1991).

2.8. Protein quality

The term 'protein quality' typically relates to both the availability of the essential AA, as well as the inherent digestibility of the particular protein using *in vitro* or *in vivo* digestibility methods. Amino acids can be divided into three categories based on their indispensability in the human body (Mercer et al. 1989): a) *indispensable or essential AA*, which include histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine; b) *conditionally indispensable AA*, which include arginine, cysteine and tyrosine (Note, these may be considered essential for infants or people with compromising health conditions); and c) *dispensable AAs* (Laidlaw and Kopple, 1987). Protein digestibility varies between pulses and cereals, where cereals digest better than pulses due to their lower levels of anti-nutritional factors

present. Some common techniques found in the literature for *in vitro* and *in vivo* testing of protein digestibility are summarized in Tables 2.1 and 2.2, respectively.

In the case of the former, assays are typically done on the benchtop and tend to be less expensive than the *in vivo* assays. Previous studies include enzymatic digestion, near-infrared spectroscopy, and advanced simulation technique. One of the most common technique is enzymatic hydrolysis methods, which involves incubation with enzyme(s) to hydrolyze amino acids and measurement of the change in pH. Enzymatic digestion methods can be further classified by the procedure and the apparatus required. The one-step incubation method uses pepsin, papain or pronase (Buchanan, 1969; Moughan et al., 1989). This method is simple and showed sufficient information regarding comparison of different treatments on the same sample, but since only one enzyme was involved, the ability to release amino acids was limited and may show low correlation with *in vivo* results (Moughan et al., 1989; Moughan, 1999). The two-stage digestion method usually involves pepsin followed by pancreatin incubation to simulate both the gastric and small intestine, which is expected to be more accurate and better correlates with the *in vivo* results. Akeson and Stahmann (1964) validated the IVPD from animal and plant sources using two-stage digestion method with the *in vivo* biological value and their correlations were higher than 0.98. Saunders et al. (1973) also reported a correlation of 0.87 between two-stage IVPD and *in vivo* protein digestibility. Later on, Furuya et al., (1979) modified this method by digesting intestinal fluid from pigs instead of pancreatin, following the pepsin-HCl digestion and reported a correlation of 0.98. Hsu et al. (1977) proposed a multi-enzyme system using a mixture of multiple enzymes for digestion and measure the pH drop after 10 min of digestion. In their study, they used trypsin, chymotrypsin and peptidase mixture for *the in vitro* analysis and conducted the *in vivo* digestibility (apparent digestibility) on rats. They then obtained the regression equation $Y = 210.46 – 18.10X$ (where Y represented the *in vivo* protein digestibility and X represented the pH after 10 min digestion) with high correlation ($r = 0.9$) and later modified by Pedersen and Eggum (1983) by keeping the pH constant using NaOH titration to avoid the limitation of buffer capacity. This is a simple and fast method. It showed a good correlation with *in vivo* digestibility and good reproducibility across laboratories (McDonough et al., 1990). The equation was later modified to $Y = 65.66 + 18.10 X$ (where Y represented the *in vivo* protein digestibility and X represented the pH change within 10 min digestion) to adapt to the situation where the starting pH was not exactly 8.0 (Tinus et al., 2012). However, in the mathematical aspect, a digestibility over 100% is possible

Table 2.1 Existing *in vitro* assays found in the literature for measuring protein digestibility.

Method	Description	Correlation with *in vivo* results	Advantages and limitations	Ref
One-step incubation	Sample incubated with single enzymes, e.g., papain or pronase	IVPD lower than *in vivo* results	Effective in comparing the effects of treatments on the same food product; Correlation with *in vivo* results varies.	1
Two-stage digestion	Simulation of gastric and intestinal digestion. Sample digested by pepsin-HCl solution first and then digested with pancreatin, trypsin or intestinal fluid after neutralization	$r > 0.8$	Good reproducibility and acceptable accuracy; Small sample size required; Good for preliminary study; Accuracy affected by fibre content.	2, 3, 4
Multi-enzyme system	Samples incubated with multiple enzymes (e.g., trypsin, chymotrypsin, peptidase, and *Streptomyces griseus* protease) and the pH drop after digestion is used for analysis	$r > 0.9$	Good correlation with *in vivo* results; Expresses the effect of processing and sample type; Not suitable for complexed food materials and accuracy may be affected by buffering capacity.	5
Constant pH by titrating NaOH (pH-stat)	The digest pre-digested by pepsin and kept at constant pH by adding 0.1 M NaOH and the amount of NaOH consumed is used for analysis	$r > 0.95$	Good correlation with *in vivo* results and good reproducibility.	6, 7
Near-infrared spectroscopy (NIRS)	Sample scanned by spectrophotometer and optical data were used for calibration and validation	Vary ($r^2 = 0.23 – 0.76$)	Rapid and low cost; A large number of *in vivo* reference data is required for calibration.	8,9
TIM	A computer-controlled model with multiple compartments that simulates gastric, intestinal and large bowel activities.		Advanced and sophisticated; Able to simulate gastrointestinal conditions of different species and ages; Expensive and difficult to operate. Validity is not yet been fully tested.	10

[1] Buchanan (1969); [2] Akeson and Stahmann (1964); [3] Saunders et al. (1973); [4] Furuya et al. (1979); [5] Hsu et al. (1977); [6] Pedersen and Eggum (1983); [7] Salazar-Villanea et al. (2016); [8] Garnsworthy et al. (2000); [9] Hervera et al. (2009); [10] Havenaar et al. (2013).

Table 2.2 *In vivo* methods for protein quality (Bender and Doell, 1957; Boye et al., 2012; FAO/WHO, 2013; Sikorski, 2001).

Methods	Equation	Summary	Limitation
Amino acid score (AAS)	AA content in 1g of test protein / the same AA content in 1g of reference protein	Focuses on four most commonly limited AA (Lys, sulphur AA, Trp, and Thr).	Fails to provide information on AA bioavailability; Does not take processing and length of the peptide into account.
Nitrogen balance (NB)	Nitrogen intake - nitrogen excreted	Indicates body nitrogen retention and measure available dietary protein intake for organisms.	The period between the intake and output is long, which is time-consuming.
Protein efficiency ratio (PER)	Weight gain/protein consumed	Most widely used method to measure the weight growth of rats caused by protein intake.	The requirement of AA for rats is different from human, resulting in underestimating some plant proteins and overestimating some animal origin proteins; Results may be affected by levels of protein taken.
Net protein ratio (NPR)	(Weight gain + weight loss of non-protein diet rats) / protein consumed	Is similar to PER but take the weight loss of non-protein diet rats into consideration.	The same as PER.
Protein rating (PR)	PER*reasonable daily protein intake	An official method of Health Canada.	The same as PER.
Biological value (BV)	Retained nitrogen / absorbed nitrogen	Determines how well the amino acids in the target protein match the required.	Results are significantly affected by the level of nitrogen intake.

Methods	Equation	Summary	Limitation
Net protein utilization (NPU)	(0.16*(24 hour protein intake in grams)-(24 hour urinary urea nitrogen)- 2-0.1 *(ideal kg of body weight))/(0.16*(24-hour protein intake in grams)	A simple measurement of the nitrogen retained for processed food.	Accuracy is limited by the protein content in the diet.
Protein digestibility corrected amino acid score (PDCAAS)	100* [(mg of digestible dietary limiting amino acid in 1 g of the test protein) / (mg of the same amino acid in 1 g of reference protein)] * true fecal digestibility (%)	Expresses the effects of protein content, AA composition and protein digestibility.	Truncation underestimates the value of good protein sources; Fecal digestibility may overestimate protein digestibility; Bioavailability is not included in the determination.
Digestible Indispensable Amino Acid Score	100* [(mg of digestible dietary indispensable AA in 1g of the dietary protein) / (mg of the same dietary indispensable AA in 1 g of the reference protein)]	A more accurate method modified from PDCAAS. Includes the effects of age, ileal digestibility and bioavailability.	Requires a robust dataset; Human test is needed to justified the results.

in food products if the pH change is higher than 1.90 and no *in vitro* protein digestibility would be lower than 65.66. Some studies applied near infrared spectroscopy (Garnsworthy et al., 2000; Hervera et al., 2009) but this method requires a large number of data for calibration and the correlation with *in vivo* results varied. A more advanced technique using a computer-controlled, multi-compartmental *in vitro* gastrointestinal system (TIM) has been applied to studies on the digestion of food, and availability and absorption of nutrients and drugs (Havenaar et al., 2013). This model is able to closely simulates the gastric, small intestinal and large bowel activity of adults, infants and animals (Butts et al., 2012). However, it is obvious that the cost of operation and maintenance will be much higher than other methods. Overall, there are numerous approaches that do not require an animal test to investigate the protein digestibility. Among various approaches, enzymatic assays of protein digestibility are rapid, simple, relatively accurate and need a small amount of sample. Therefore they are recommended for preliminary studies and are commonly used methods to obtain a general understanding of the protein digestibility of a sample.

Although possessing many advantages, *in vitro* results must be correlated with *in vivo* data. *In vivo* assays involve the use of animals (e.g., pig, rats etc.), typically expensive, labour-intensive and time-consuming. The system of protein claims for foods differs in different areas such as European Union, Canada and the United States. In Canada, a protein rating is required and the protein rating higher than 20 is required for a "source of protein" claim of the food product (Canadian Food Inspection Agency). However, the most widely accepted assay by the international community (i.e., United Nations Food and Agriculture Organization (FAO)) is the Protein Digestibility Corrected Amino Acid Score (PDCAAS), which is now being recommended to be replaced by the Digestible Indispensable Amino Acid Score (DIAAS) (Schwenke, 2001; Vaz Patto et al., 2015). The PDCAAS was recommended by the FAO/WHO in 1989 as a method to assess the protein quality, taking both the digestibility and composition of AA into account. This score is measured based on the AA requirement pattern of preschool children (Sarwar, 1997). It is calculated according to the following equation:

PDCAAS (%) = 100* [(mg of digestible dietary limiting amino acid in 1 g of the test protein) / (mg of the same amino acid in 1 g of reference protein)] * true faecal digestibility (%) (Eq. 2.1)

This equation takes the limiting amino acid into consideration and provides a comparison to how well the test protein is uptake relative to the ideal protein (e.g., casein). However, the PDCAAS is limited to values between 0 and 1, and as such depending on the source and food matrix effects, values can be truncated (Schaafsma, 2000). Since this equation is based on the fecal digestibility, it is assumed that the digestibility of crude protein and certain AA are similar (FAO/WHO, 2013). In addition, the PDCAAS method does not take into account the anti-nutritional factors present (Sarwar, 1997). Consequently, PDCAAS is only accurate in evaluating foods that contain a small amount of anti-nutritional factors that are highly digestible (Sarwar, 1997).

Based on the limitations of the PDCAAS method, the Digestible Indispensable Amino Acid Score (DIAAS) is now being recommended by the FAO as the new gold standard for measuring protein quality (Leser, 2013). AA scoring patterns used are based on nutritional requirements for specific age categories, such as infants (newborn to 6 months old), young children (6 months to 3 years old) and older children or adults (3 years plus). DIAAS values are calculated based on this following equation (FAO/WHO, 2013):

DIAAS (%) = 100* [(mg of digestible dietary indispensable AA in 1g of the dietary protein) / (mg of the same dietary indispensable AA in 1 g of the reference protein)]

(Eq. 2.2)

The DIAAS method made various improvements to the PDCAAS test, such as:

- *Inaccuracy in certain proteins:* Since the PDCAAS is based on true fecal digestibility, values can be greater than the true digestibility of protein; digestion will be impacted by microbes within the gastrointestinal tract. In the DIAAS method, ileum digestibility is used, and focuses on individual AA digestibility rather than crude protein digestibility, making the overestimation and underestimation less likely to happen (Leser, 2013).
- *Truncation of scores*: The DIAAS method does not have the same upper score limitation of 1, as the PDCASS method, allowing better separation of the high-quality proteins since the truncation of values does not occur (Leser, 2013).
- *Bioavailability*: The DIAAS method uses reactive lysine rather than total lysine in the calculation, thereby allowing protein damage caused by processing to be taken into

consideration to give a better estimate of bioavailability (Leser, 2013).

Current issues related to using the DIAAS methods are related to insufficient databases for true ileal digestibility, as such more human and animal studies are required in order to accelerate the application of DIAAS.

As an important nutritional and energy compound, protein content is required in nutritional labelling. Moreover, according to the Food and Drug Regulation in Canada, a claim of "source of protein" is allowed provided the food has a protein rating no less than 20 and of "excellent source of protein" is allowed provided the food has a protein rating no less than 40. And in order to obtain the protein rating, a PDCAAS or a PER is required. As such, PDCAAS is still an official means to assess protein quality of food products. For food labelling purposes, the protein rating of a food product with one protein source can be calculated by multiplying the PER of a food by Reasonable Daily Intake. When it comes to a food product with multiple protein sources, the estimated PER was calculated by using the estimated PDCAAS multiplied by 2.5. In brief, estimated PER or PER determines the protein claim of the final products provided the products belong to the same category, i.e., have the same reasonable daily intake value. By calculating the estimated PER or PER, the protein claim of a certain product could be preliminarily evaluated.

Table 2.3 PDCAAS, PER and protein rating of pulses, cereals and casein.

Source	Processing condition	PDCAAS	PER	DIAAS	Protein rating	Ref
Red kidney beans	Soaked/Boiled	0.55	1.55	0.51	23.98	1
	Raw	-	2.95	-	-	2
Navy beans	Soaked/Boiled	0.67	1.51	0.65	24.53	1
Whole green lentils	Soaked/Boiled	0.63	1.30	0.58	18.29	1
	Extruded	0.57	1.08	0.53	-	3
	Cooked	0.53	0.98	0.49	-	3
	Baked	0.47	0.88	0.44	-	3
Split red lentils	Soaked/Boiled	0.54	0.98	0.50	18.31	1
	Extruded	0.63	1.05	0.58	-	3
	Cooked	0.57	1.14	0.53	-	3
	Baked	0.54	0.79	0.50	-	3
Split yellow peas	Soaked/Boiled	0.64	1.42	0.73	20.04	1
	Raw	-	2.58	-	-	2
Split green peas	Soaked/Boiled	0.50	0.86	0.46	13.17	1
Black beans	Soaked/Boiled	0.53	1.61	0.49	24.55	2
Kabuli chickpeas	Soaked/Boiled	0.52	2.32	0.67	30.44	1
Pinto beans	Soaked/Boiled	0.59	1.64	0.60	23.27	1
	Extruded	0.58	1.47	0.57	-	4
Buckwheat	Milled	0.55	2.55	0.54	-	4
	Extruded	0.86	2.62	0.63	-	4
Casein	Raw	1.00	-	1.31	-	1
Barley	Air dried	0.49	1.7	-	-	5
Wheat	Raw	0.45	0.8	-	-	5
Oat	Raw	0.71	1.8	-	-	5
Millet	Pearl, raw	0.21	-	-	-	5
Maize	Raw	0.41	1.4	-	-	5

[1] Nosworthy et al. (2017b); [2] Khattab et al. (2009); [3] Nosworthy et al. (2018); [4] Nosworthy et al. (2017a); [5] Boye et al. (2012)

3. MATERIALS AND METHODS

3.1. Materials

Desi chickpeas (var.: CDC Consul, dehulled and split, grown in Elbow, SK in 2014) and hull-less barley (var.: CDC McGwire grown in Star City, SK in 2014) were purchased for this study from Diefenbaker Seeds (Saskatoon, SK) and InfraReady Product (1998) Ltd. (Saskatoon, SK), respectively. All seeds were stored dry, in large plastic sealed containers at room temperature (21-23^{o}C). All chemicals were purchased from Sigma-Aldrich (Oakville, ON, Canada) unless otherwise specified and were of reagent grade. The water used in this research was produced from a Millipore Milli-Q^{TM} water purification system (Millipore Corp., Milford, MA, USA).

3.2. Preparation of flour materials

In this study, chickpeas and barley grains were left un-tempered or tempered to 20% moisture prior to infrared heating. For tempering, ~ 8 kg of seeds were placed in sealed polyethene bags containing Milli-Q water, at the amount specified by the following equation based on initial seed moisture according to AACC Official Method 26-95.01 (AACC, 1999):

$$W(H_2O) = \frac{W_S \times [\text{Moisture}_E - \text{Moisture}_O]}{100 - \text{Moisture}_O} \qquad \text{[Eq. 3.1]}$$

where Ws represents the weight of samples used for tempering, M_o (Chickpea: M_o = 8.27 %; barley: M_o = 8.60 %) and M_E (~20%) are original and end moisture levels (%) found in un-tempered and tempered seeds, respectively. The tempering was carried out at room temperature and atmospheric pressure. A preliminary moisture uptake experiment was conducted over time (0.5 to 8 h) to find an equilibrium for both seeds, which was reached after 1 h at the 20% moisture level.

Infrared heating was carried out at InfraReady Product (1998) Ltd. (Saskatoon, SK) using a laboratory scale micronizer (Model A 156379 –B0, FMC Syntron ® Bulk Handling Equipment, Homer City, PA, USA). The micronizer was made of a burner (Model type R 1603-2 pat, Rinnai,

Japan) to generate heat, a Syntron feeder (Model F010, Riley Automatic Ltd., Derby, England) to feed and control the volume of processing seeds, and a Syntron magnetic feeder (Mode BF2 A, FMC Corporation, Homer City, PA, USA) to convey seeds passing the heating area. The burner was 19 cm above the conveyor and the magnetic feeder was 152 cm long. The surface temperature of the seeds was monitored using a hand-held IR thermometer (Oakton, Vernon Hills, IL, USA). Approximately 2 kg of each tempered and un-tempered sample was processed in order to reach the surface temperatures of 115 and 135^{o}C. Each heating treatment was independently carried out under the same condition three times to achieve three processing replicates. All processed seeds were dried to moisture levels <10% using the laboratory scale micronizer. Un-tempered and unheated seeds served as another control.

All seeds were then ground into coarse flour using a disc mill (Glen Mills Inc., Clifton, NJ, USA) and then into finer flour using a UDY Cyclone Sample Mill with 1 mm mash (UDY Corporation, Fort Collins, CO, USA). Flours were stored in polyethene Ziploc bags at 4^{o}C in a cold room.

In the case of blends, only the chickpea and barley flours tempered to 20% moisture and heated to a seed surface temperature of 135^{o}C (from one processing run) were used based on protein quality and anti-nutritional results (See Section 4.2). One barley and one chickpea sample under this treatment were selected and the flours were blended in polyethene bags by hand mixing at six ratios (w/w) of chickpea to barley at 0: 100, 20: 80, 40: 60, 60: 40, 80: 20 and 100: 0. The flours were stored under the same condition as the original flour. Blends were analyzed for their proximate composition, functionality and protein quality. Data were reported as the mean from three independent measurements ± standard deviation (n = 3) following the same methods mentioned above.

3.3. Physicochemical properties

(a) Proximate analysis

Proximate analysis of all flours was carried out according to the Association of Official Analytical Chemists (AOAC) methods 925.10, 923.03, 920.85 and 984.13A for moisture, ash, crude fat and crude protein (%N x 6.25 for chickpeas and blended flours and 5.7 for barley flour), respectively (AOAC, 2003). Ash, fat and protein levels are reported on a percent dry weight basis

(d.b.). For all composition analyses, measurements were made in duplicate on triplicate processing samples, and reported as the mean ± one standard deviation (n = 3).

(b) Total starch content

The total starch content of all flours was determined according to the American Association of Cereal Chemists official method 76-13.01 (AACC, 1999). The assay involved using the Megazyme total starch assay kit (Megazyme International Ireland Ltd., Bray, Ireland). The kit contained thermostable α-amylase (5 mL, 3,000 U/mL on Ceralpha reagent* at pH 6.5 and 40°C), an amyloglucosidase (5 mL, 3,300 U/mL on soluble starch or 200 U/mL on p-nitrophenyl β-maltoside) solution, a glucose determination (GOPOD) reagent buffer, GOPOD reagent enzymes, a 1.0 mg/mL d-glucose standard solution and a standardized regular maize starch with a starch content of 93% (on dry weight basis). The thermostable α-amylase was diluted with 30 mL of sodium acetate buffer (100 mM pH 5.0) for later use. The GOPOD reagent buffer was diluted with 1 L of water and the resulting dilution was used for dissolving the GOPOD reagent enzymes to prepare a GOPOD reagent. In brief, a starch solution containing 0.1 g standardized regular maize starch was prepared as a standard each time the assay was prepared. The maize starch was dispersed in 0.2 mL 80% (v/v) ethanol and incubated with 3 mL of a thermal stable α-amylase solution in boiling water for 6 min with vortex mixing at 2 min intervals. Then 0.1 mL of amyloglucosidase was added and mixed. The flour samples were pre-washed with 80% (v/v) ethanol, followed by centrifugation at 1,800 x *g* using a VWR Clinical 200 centrifuge (VWR International, Mississauga, ON) for 10 min at 83^{o}C for 5 min to remove the pre-existing d-glucose and maltodextrins. The supernatant was discarded, and the pellet was resuspended in 2 mL of 2 M KOH to dissolve the resistant starch. The pellet-KOH solution was then placed in an ice/water bath for 20 min. Eight mL of 1.2 M sodium acetate buffer (pH 3.8) was then added after removal from the ice/water bath. Then 0.1 mL of the thermostable α-amylase solution and 0.1 mL amyloglucosidase was added to the solution followed by incubation at 50^{o}C for 30 min in a water bath. The digested solution, along with the standard maize solution was transferred to a 100 mL volumetric flask and volume adjusted with water. Aliquots of the diluted solutions were then transferred to centrifuge tubes and then centrifuged at 1,050 x *g* for 10 min. One hundred μL of the supernatant was then transferred into glass tubes in duplicate and incubated with 3 mL of the glucose determination reagent (containing glucose oxidase, peroxidase and 4-aminoantipyrine) at

50°C for 20 min in a water bath prior to measuring absorbance at 510 nm using a UV-VIS spectrophotometer (Genesys 10-S, Thermo Scientific, Madison, WI, USA). A reagent blank and a d-glucose standard solution were also prepared. One hundred µL of the reagent blank containing 0.1 mL water and d-glucose standard containing 0.1 mL of 1.0 mg/mL d-glucose standard solution were then each incubated with 3 mL of glucose determination reagent at 50°C for 20 min in a water bath prior to measuring the absorbance. Total starch levels were calculated using Mega-Calc™ worksheet provided by Megazyme (Megazyme International Ireland Ltd., Bray, Ireland) following the eq. 3.2.

$$\text{Starch}, \% = \Delta A \times FV \times \frac{F}{W} \times 0.9 \qquad \text{[Eq. 3.2]}$$

where, ΔA is the absorbance of the sample reading against reagent blank at 510 nm; FV is the final volume after dilution (100 mL); F is the conversion factor converting absorbance to µg of glucose (F = 100 mg of glucose used/ absorbance of that glucose solution); W is the weight of the flour used. The measurements were made in duplicate on triplicate processing samples, and reported as the mean ± one standard deviation (n = 3).

(c) Gelatinized starch

The gelatinized starch content of chickpea and barley flour was determined using the method proposed by Chiang and Johnson (1977) and modified by Emami et al. (2010b) and Pathiratne et al. (2015). In brief, 20 mg of flour was weighed and mixed with 5 mL of 80% (v/v) ethanol in a 50-mL centrifuge tube. The suspension was then kept in water bath at 40°C for 10 min before centrifugation at 1,500 x g for 10 min (VWR Clinical 200 centrifuge, VWR International, Mississauga, ON). After the supernatant was discarded, the pellet was resuspended in 5 mL of 80% (v/v) ethanol followed by another 5 mL of ethanol with vortex mixing. The suspension was then centrifuged at the same condition and the supernatant was discarded. The resulting pellet was then dried in the oven at 30°C overnight until it was completely dried. Then 5 mL water and 25 mL amyloglucosidase solution (2 g of 60,000 unit/g amyloglucosidase from *Aspergillus Niger* with 250 mL pH 4.5 sodium acetate buffer) was added to the pellet and vortexed to avoid clumping and they were incubated in a water bath for 30 min at 40°C with consistent shaking. After 30 min, 2 mL of 25% (w/v) trichloroacetic acid were added and then the mixture was centrifuged at 1,500 x

g for 5 min (VWR Clinical 200 centrifuge, VWR International, Mississauga, ON) and the supernatant was kept. A hundred μL of solution was taken from 0.25, 0.5, 0.75 and 1 mg/mL of standard glucose solutions along with the same amount of supernatant for analysis. Then 3 mL of *o*-toluidine reagent prepared by dissolving 1.5 g of thiourea in 940 mL of glacial acetic acid and 60 mL *o*-toluidine were added to each solution and the mixture was heated in boiling water for 10 min. The absorbance of the resulting solution was measured at 630 nm after cooling down to room temperature. A glucose vs. absorbance standard curve was made and the glucose content in the sample was calculated from the standard curve. The starch content of the samples was calculated from glucose content on a dry basis as glucose × 0.9. The gelatinized starch content (G) was calculated according to the following equation:

$$G\ (\%) = \frac{D-K}{Total\ starch} \times 100 \qquad \text{[Eq. 3.3]}$$

where, D stands for the starch digested by amyloglucosidase in the sample, which was calculated by glucose × 0.9. K stands for the correction factor, which was the slope of glucose released vs. the amount of the un-tempered and un-heated chickpea or barley flour (mg) multiplied by 100. The measurements were made in duplicate on triplicate processing samples, and reported as the mean ± one standard deviation (n = 3).

(d) Colour

The colour of all treatments of flour was measured using a Hunterlab MiniScan XE colourimeter (Hunter Associates Laboratory, Inc., Reston, VA). The three coordinates, L*, a* and b* were measured. The L* represents the lightness (0 = black, 100 = white); a* indicates the degree of red-green (-a = greenness, +a = redness); and b* represents the degree of yellowness (-b = blueness, +b = yellowness). A standard white tile $L_s = 92.81$, $a_s = -1.25$, $b_s = 1.04$ was used for calibration. All measurements were made in duplicate on triplicate processing samples, and reported as the mean ± one standard deviation (n = 3).

(e) Surface charge (zeta potential)

The surface charge was measured following Chang et al. (2015) by determining the electrophoretic mobility (U_E) in a 0.05% (w/w) protein solution at pH 7.0 using a Zetasizer Nano-

EZ90 (Malvern Instruments, Westborough, MA, USA). Twenty-five mg of flour were weighed and dispersed in water to make a total weight of 50 g (0.05%, w/w). The solution was adjusted to pH 7.0 with 0.1 N of HCl or NaOH before stirring overnight and the pH was adjusted to pH 7.0 again before determination. The surface charge (ξ) was calculated following Henry's equation:

$$U_E = \frac{2\varepsilon \times \xi \times f(\kappa\alpha)}{3\eta} \quad \text{[Eq. 3.4]}$$

where ε (Farad/m) refers to the permittivity, *f*(κα) is Smoluchowski approximation, a function that is set to 1.5 in this study relating to the Debye length (κ) and the ratio of particle radius (α), and η (mPa·s) is the dispersion viscosity.

3.4 Functional properties

(a) Protein solubility

Protein solubility of chickpea and barley flours was determined by measuring the nitrogen content dissolved in water at pH 7.0 according to Morr et al. (1985) and Liu et al. (2010). In brief, 0.2 g of flour was accurately weighed and dissolved in 18.0 g of water to prepare a 0.1% (w/w) solution. The solution was adjusted to pH 7.0 with 0.1 N HCl or NaOH and stirred for 1 h to further dispersion. The pH of the solution was measured during this process to ensure proteins were dissolved at required pH value. The weight of the flour-water mixture was adjusted to 20.0 g with water after 1 h of mixing. The mixture was left standing for 10 min for undissolved particles to precipitate. Then an aliquot from the upper layer of the mixture was transferred to a centrifuge tube and centrifuged at 4,430 x *g* for 10 min at room temperature. Then 5.0 g of supernatant were accurately weighed to a micro-Kjeldahl digestion flask and the nitrogen content was determined following the same procedure as crude protein (AOAC, 2003). The protein concentration in the supernatant was calculated by the following equation using a nitrogen conversion factor of 6.25 for chickpea (and chickpea: barley blends) and 5.70 for barley flour:

$$\text{Protein concentration in the supernatant (\%)} = \frac{Volume\ of\ HCl\ consumed\ (mL) \times Normality\ of\ HCl \times 14.007 \times 100}{weight\ of\ supernatant\ (g) \times 1000} \times N\ factor$$

[Eq. 3.5]

Protein solubility was calculated by the following equation:

$$\text{Protein solubility (\%)} = \frac{20.0 \times protein\ concentration\ in\ the\ supernatant\ (\%)}{Weight\ of\ the\ flour\ (g) \times protein\ concentration\ of\ flour(\%)} \times 100$$

[Eq. 3.6]

(b) Water hydration capacity (WHC)

Water hydration capacity was measured according to the AACC Method 56-20.01. In brief, 1 g of flour (as-is) was accurately weighed and mixed with 20 mL distilled water by a Vortex mixer (VWR, Mississauga, ON, Canada) for 10 s

every 5 min, followed by centrifugation at 1,000 x *g* for 15 min using a VWR Clinical 200 centrifuge (VWR International, Mississauga, ON). The supernatant was decanted and the weight of the residue was recorded. The WHC is calculated according to eq. 3.7 and reported as g water/g flour on dry basis.

$$\text{WHC} = \frac{\text{weight of residue} - \text{weight of sample}}{\text{sample weight (dry basis)}} \quad \text{[Eq. 3.7]}$$

(c) Oil holding capacity (OHC)

OHC was determined according to Nidhina and Muthukumar (2015) with slight modifications. In brief, 1.0 g (W_0) of accurately weighed flour (as is) was added to 10 g of refined canola oil (Great Value^{TM} purchased at a local supermarket) within a 50-mL centrifuge tube, and then mixed for 10 s using a Vortex mixer (VWR, Mississauga, ON, Canada) every 5 min for 30 min. The samples were then centrifuged for 15 min at 1,000 × g at room temperature (VWR, Mississauga, ON, Canada). The supernatant was removed and the pellet was weighed and recorded as W′. The OHC was calculated using following eq. 3.8 and reported as g oil/g flour on a dry basis.

$$\text{OHC} = \frac{W' - W_0}{W_0} \quad \text{[Eq. 3.8]}$$

(d) Foaming capacity (FC) and stability (FS)

The foaming properties were determined according to Wilde and Clark (1996). In brief, 1% (w/w) flour solutions were prepared and adjusted to pH 7.0 using 1 N NaOH before stirring overnight (~16 h) at room temperature to facilitate increased protein solubility. The solution pH was adjusted again to 7.0 prior to analysis. Fifteen mL of this solution were transferred into a 400-mL beaker and then homogenized using an Omni Macro Homogenizer (Omni International, Marietta, GA, USA) equipped with a saw tooth probe at speed 4 for 5 min. The fixture blade was placed slightly below the air-water interface to generate the most foam. The sample was then quickly transferred into a 50-mL graduated cylinder and the volume of the foam was recorded as V_1. After 30 min, the volume of the foam was measured and recorded as V_2. The foaming capacity and stability were calculated based on the following eq. 3.9 and eq. 3.10:

$$\% \text{ FC} = \frac{V_1}{15\ mL\ Initial\ Volume} \times 100\% \qquad \text{[Eq. 3.9]}$$

$$\% \text{ FS} = \frac{V_1 - V_2}{V_1} \times 100\% \qquad \text{[Eq. 3.10]}$$

(e) Emulsion activity (EA) and stability (ES)

The emulsifying properties were determined according to Yasumatsu et al. (1972). Both the emulsion activity and emulsion stability were determined. In brief, 3.5 g of flour was dispersed in 50 mL of water. The aqueous flour solution (50 mL) was then added to 50 mL canola oil and homogenized using an Omni Macro Homogenizer (Omni International, Marietta, GA, USA) at speed 4 for 1 min. The emulsion was then transferred into two 50 mL centrifuge tubes and centrifuged at 1,300 x g for 5 min using an Allegra X-22 Series (Beckman Coulter Inc., Mississauga, ON) centrifuge. The height of the emulsified layer and the entire emulsion in the tube was then recorded by using a ruler. EA was determined using eq. 3.11.

$$\% \text{ EA} = \frac{Height\ of\ the\ emulsified\ layer\ (cm)}{Height\ of\ the\ entire\ layer\ in\ tube\ (cm)} \times 100\% \qquad \text{[Eq. 3.11]}$$

The same emulsion was then heated for 30 min at 80^{o}C within a water bath and then cooled to room temperature (22-23^{o}C) within a second water bath for 15 min. The heat-treated emulsion was

then divided into four tubes (~12.5 mL) and centrifuged at 1,300 x *g* for 5 min using the same centrifuge. The height of the emulsified and the entire emulsion was then recorded. EA was determined using eq. 12.

$$\text{ES} = \frac{Height\ of\ the\ emulsified\ layer\ (cm)}{Height\ of\ the\ whole\ layer\ in\ tube\ (cm)} \times 100\% \qquad \text{[Eq. 3.12]}$$

(f) Pasting properties

The pasting properties of chickpea and barley flours were determined according to the AACC method 76-21.01 (1999) following the setting of STD 2 using an AR-G2 Magnetic Bearing Rheometer (TA Instruments Ltd., West Sussex, England) with a starch pasting cell. In brief, 3.5 g flour with 14% moisture content was used in this experiment. The weight of flour (S) with different moisture content was calculated based on eq. 3.13 and the weight of the water (W) was adjusted accordingly based on eq. 3.14. The flour-water mixture was placed in the test canister and stirred by a plastic paddle at 160 rpm. The temperature was increased and kept at 50^{o}C for 1 min before raising the temperature to 95^{o}C in 7.5 min. The temperature was then held at 95^{o}C for 5 min before reducing to 50^{o}C in 7.5 min. Then the temperature was held at 50^{o}C for 2 min. The viscosity was automatically recorded every 4 s during the entire process. The pasting curve of viscosity (Pa·s) vs. time (min) was plotted.

$$\text{S} = \frac{86 \times 3.5}{100 - \text{Moisture}(\%)} \qquad \text{[Eq. 3.13]}$$

$$\text{W} = 25 + 3.5 - \text{S} \qquad \text{[Eq. 3.14]}$$

The peak viscosity, trough, breakdown, final viscosity, setback, peak time and pasting temperature were determined.

3.4. Anti-nutritional properties

For all anti-nutritional compound analyses, measurements were made in duplicate on triplicate processing samples for the chickpea and barley flours only (i.e., no blends), and reported as the mean ± one standard deviation (n = 3).

(a) Total phenolic content

The total phenolic content of the flours was determined according to Singleton and Rossi (1965), and Chiremba et al. (2009). This method uses spectrophotometry to determine the change of absorbance of phenolic groups due to their reaction with Folin Ciocalteu reagent. In brief, 2 g of the flour treatments were extracted with 5 mL of 1% HCl in methanol by vortexing for 15 s every 20 min for 2 h (Vortex mixer, VWR International, Mississauga, ON, Canada), followed by centrifugation at 1,050 x *g* (VWR Clinical 200 centrifuge, VWR International, Mississauga, ON) for 10 min to recover the supernatant. This extraction procedure was repeated 2 more times and the supernatant from each time of extraction was pooled for later analysis. A standard catechin (monohydrate (+)-catechin, Catalog No. ALX-385-017-G001, Enzo Life Sciences Inc., Farmingdale, NY) curve was obtained by preparing a 1 mg/mL catechin stock solution, followed by diluting with water to obtain catechin concentrations of 0, 0.2, 0.4, 0.6, 0.8 and 1 mg/mL. Five hundred μL of each standard concentration was transferred to a 50 mL volumetric flask with 2.5 mL of Folin Ciocalteu phenolic reagent. Then 7.5 mL 20% (w/w) Na_2CO_3 was quickly added and brought to volume with water. The samples were determined in duplicates by using 0.5 mL of sample extract instead of 0.5 mL of catechin dilutions and following the same procedure. The absorbance was measured at 760 nm using a spectrophotometer (Genesys 10-S, Thermo Scientific spectrophotometer, Madison, WI, USA) exactly 2 h after the addition of Na_2CO_3. The total phenol content was calculated using eq. 3.15, and the results were expressed per mg of catechin equivalent/ g of flour on a dry basis.

Total phenolic content (g catechin equivalent / g flour) =

[(Abs of sample – y-intercept of the standard curve) / slope of standard curve] x 15 (mL of extract / 2 (gram of flour)

[Eq. 3.15]

(b) Condensed tannins

The level of condensed tannins within the flours was determined according to the vanillin assay reported by Price et al. (1978). A working vanillin reagent for analysis was prepared daily by mixing the stock solution of 1% vanillin in methanol and 8% HCl in methanol at the volume ratio of 1:1. In brief, 0.2 g of each sample was extracted using 10 mL absolute methanol for 20

min followed by centrifugation at 3,000 x *g* for 10 min with a VWR Clinical 200 centrifuge (VWR International, Mississauga, ON). A 0.3 mg/mL of catechin solution was prepared every day by dissolving 3.0 mg catechin in 10 mL methanol. A standard curve was made by making a serial dilution using that catechin solution. Zero, 0.1, 0.2, 0.3, 0.4 and 0.5 mL of the catechin solution were transferred to a set of six tubes and volume adjust to 1 mL with methanol. Another set of tubes was prepared in the same way. Both sets of tubes were incubated in a 30°C water bath. Five mL of the working reagent was added to one set of tubes and 5.0 mL of 4% HCl was added to the other set of tubes in 1 min interval. The two sets of tubes were incubated at 30°C for exactly 20 min and then the absorbance was measured at 500 nm using a spectrophotometer (Genesys 10-S, Thermo Scientific spectrophotometer, Madison, WI, USA). The standard absorbance vs. mg catechin curve was made according to the results obtained. One mL of the supernatant of samples were transferred into 3 tubes and two of them were allowed to react with 5 mL of working reagent and the third one incubated with 5 mL of 4% HCl using the same procedure described above. The level of condensed tannins was determined using eq. 3.16, and the results were expressed on per gram of flour on a dry basis.

$$\text{Tannins (mg of catechin equivalent per g of flour)} = \frac{Abs_1 - Abs_0 - b}{a} \times 10\ mL\ of\ extract \div 0.2\ g\ of\ sample \qquad \text{[Eq. 3.16]}$$

where Abs_1 refers to the absorbance of samples with working reagent, Abs_0 refers to the absorbance of the solution with HCl, *b* refers to the intercept of the standard curve and *a* refers to the slope of the standard curve.

(c) Trypsin inhibitor activity

The trypsin inhibitor activity was determined according to AACC method 22-40.01 with modifications. In brief, 0.1 g of flour was weighed and extracted with 25 mL 0.01 N NaOH for 3 h, followed by centrifugation at 3,000 x *g* for 20 min at 4°C using a Sorvall RC 6+ Centrifuge (Thermo Fisher Scientific Inc., Waltham, MA, USA). The pH of the supernatant was adjusted to 9.0 using 1 N HCl. Then, 0, 0.6, 1.0, 1.4 and 1.8 mL of extract was transferred to five 15 mL centrifuge tubes, volume adjusted to 2.0 mL with water. Each tube was incubated in a water bath at 37°C with 2 mL of trypsin solution for 10 min. Five mL of Na-Benzoyl-D, L-arginine 4-

nitroanilide hydrochloride (DL-BAPNA) substrate solution pre-warmed at 37ºC were added into each test tube and then they were incubated for another 10 min, before stopping the reaction by adding 1 mL of 30% acetic acid. The solution was then filtered through Whatman No. 2 paper (GE Healthcare UK Limited, Buckinghamshire, UK). A blank was prepared for each sample concentration in a similar manner, except the acetic acid was added prior to the addition of the trypsin solution. The absorbance of the samples and the corresponding blanks were measured at 410 nm. The absorbance of the sample and blank at each concentration was measured at 410 nm using a spectrophotometer (Genesys 10-S, Thermo Scientific spectrophotometer, Madison, WI, USA). The trypsin inhibitor activity was calculated using eq. 3.17.

$$\text{TIU (mg sample)} = \frac{\text{TIU}}{\text{mL of extract taken}} \times \frac{25 \text{ mL of extract}}{100 \text{ mg of sample}} \times \text{D} \qquad \text{[Eq. 3.17]}$$

where TIU refers to the trypsin inhibitor unit, arbitrarily defined as an increase of 0.01 absorbance unit at 410 nm of the sample relative to the corresponding concentration blank. D refers to the dilution factor which preferably provides an inhibition between 40 to 60%. The result was expressed as TIU per mg of the sample and converted to dry basis.

(d) Chymotrypsin inhibitor activity

Chymotrypsin inhibitory activity within the flour was determined according to the method reported by Makkar et al. (2007). In brief, 1 g of flour was accurately weighed into a beaker and was extracted by stirring with 10 mL borate buffer (pH 7.6) for an hour. The mixture was then transferred to a centrifuge tube and centrifuged using a VWR Clinical 200 centrifuge (VWR International, Mississauga, ON) at 3,000 x g for 10 min. Zero, 0.25, 0.5 and 0.75 mL of supernatant were transferred to two sets of tubes (set 1 and 2). The volumes were adjusted to 1 mL with the borate buffer mentioned above. Then 1 mL of chymotrypsin solution was added to each tube and the tubes were incubated at 37ºC water bath for 10 min. Then 2 mL of pre-warmed casein was added to one set of tubes (set 1). The casein was prepared daily by adding 1 g of casein powder into 80 mL borate buffer (pH 7.6) and stirred at 35ºC until it was completely dissolved and the pH was kept at 7.6. The volume was brought up to 100 mL with borate buffer. The tubes with casein were incubated in a 37ºC water bath for exactly 10 min. Six mL of trichloroacetic acid reagent (18 g of trichloroacetic acid, 18 g of anhydrous sodium acetate and 20 mL of glacial acetic acid in 1 L

of distilled water) was added to each tube after incubation. Then 2 mL of casein were added to the set of tubes (set 2). All the tubes were left to stand for 30 min before filtering through Whatman No. 2 paper (GE Healthcare UK Limited, Buckinghamshire, UK). The absorbance of filtered solutions was measured at 275 nm in a quartz cuvette against the blank. One chymotrypsin unit was defined as the increase by 0.01 absorbance unit at 275 nm of the reaction mixture. The chymotrypsin inhibitory activity was calculated following eq. 3.18:

$$\text{CIU (per mg sample)} = \frac{\text{CIU}}{\text{mL of extract taken}} \times \frac{\text{10 mL of extract}}{\text{mg of sample}} \times \text{D} \qquad \text{[Eq. 3.18]}$$

where CIU was the absorbance divided by 0.01. D stands for the dilution factor, to keep the percent of inhibition within the range of 40% to 60%. The result was expressed as TIU per mg of the sample and converted to a dry basis.

3.5. Nutritional properties

(a) Amino acid composition

The amino acid composition of treated and untreated chickpea and barley flours, and blends was determined at POS Bio-Sciences Corp. (Saskatoon, SK) using a Pico-tag™ amino acid analysis system (Waters Corporation, Milford, MA, USA) and a high performance liquid chromatography (HPLC). All the 18 amino acids were determined. The determination of 15 amino acids followed the method reported by Bidlingmeyer et al. (1987). In brief, the flours containing ~20 mg protein were prepared and mixed with 15 mL 6 N hydrochloride acid in Pyrex tubes, followed by flushing with N_2. The tubes were then capped and kept at 110^{o}C for 20 h to hydrolyze the proteins into amino acids for HPLC separation and determination. The determination of tryptophan followed the AOAC method 988.15 (2005) with slight modification. The samples were first hydrolyzed by 10 M NaOH and kept in boiling water bath for 20 min and then were put in the oven at 110^{o}C for 16 h followed by HPLC determination. Tryptophan was determined by reverse phase liquid chromatography with UV detection. The concentration of sulphur amino acids, methionine, and cysteine, was determined following AOAC method 985.28 (2005) using ion-exchange chromatography with modification. The 1-octanol was not included in the procedure.

Cold performic acid was used for cysteine and methionine oxidation and they were kept for reaction at 4°C overnight. The sulphur amino acids were oxidized with performic acid and hydrolyzed with 6 M HCl at 110°C for 18 h.

(b) Determination of the amino acid score

Amino acid score refers to the ratio of 1 g of the target protein to the reference protein. The amino acid composition of the reference protein was recommended by FAO/WHO using the amino acid requirement for children 2 to 5 years of age (amino acid, mg/g protein): Histidine, 19; Isoleucine, 28; Leucine, 66; Lysine, 58; Methionine + Cysteine, 25; Phenylalanine + Tyrosine, 63; Threonine, 34; Tryptophan, 11; Valine, 35 (FAO/WHO, 1991). The amino acid score of the flour represents the most limiting essential amino acid.

(c) In vitro protein digestibility

The *in vitro* protein digestibility was determined by the pH drop of the solution digested by a multi-enzyme solution according to Tinus et al. (2012). This solution was prepared every day by mixing 31 mg chymotrypsin, 16 mg trypsin and 13 mg protease with 10 mL water and kept at 37°C. Its pH was adjusted to 8.0 ± 0.05 with 0.1 M NaOH and HCl. Approximately 0.2 g of chickpea flour or 0.6 g of barley flour (52.5 ± 0.5 mg of protein) was mixed with 10 mL water and added to 8 mL of water preheated to 50°C. The mixture was stirred for 1 h at 37°C. The pH of the solution was adjusted to 8.0 ± 0.05 with 0.1 M NaOH and HCl before adding 1 mL of the multi-enzyme solution mentioned above. The pH of the protein solution was recorded every 30 s for 10 min and the *in vitro* protein digestibility (IVPD) was calculated following the equation below:

$$IVPD\ (\%) = 65.66 + 18.10 \times \Delta pH_{10min} \quad \text{[Eq. 3.19]}$$

where $\Delta pH_{10\ min}$ refers to the change in pH from initial 8.0 to the pH after 10 min.

(d) In vitro Protein Digestibility Corrected Amino Acid Score (IV-PDCAAS)

The IV-PDCAAS was calculated as the product of the amino acid score and *in vitro* protein digestibility.

(e) Estimated PER for blended flours

The estimated PDCAAS of the blended flours was calculated according to the calculation for mixture in the FAO/WHO report (1991). The estimated PER was calculated as the estimated PDCAAS multiplied by 2.5.

3.6. Statistics

A one-way analysis of variance with a Tukey's Post Hoc test was performed to determine statistical differences between treatments [(a) un-tempered non-micronized; (b, c) un-tempered, heated to 115 or 135^{o}C; and (d, e) tempered to 20% moisture, heated 115 or 135^{o}C] for each parameter. Note: triplicate measurements were made for each parameter measured, on triplicate processing runs. The mean values from each processing run were used in the ANOVA analysis, and in the calculation of the mean ± one standard deviation (n = 3). For the un-tempered non-micronized flours (control), triplicate measurement values were used for the ANOVA analysis Blended flours with chickpea to barley ratio of 20: 80, 40: 60, 60: 40 and 80: 20 along with the original chickpea and barley flour tempered and heated at 135^{o}C were also analyzed by one-way analysis of variance followed by a Tukey's Post Hoc test. A Pearson correlation (pairwise) were conducted between significantly different physicochemical (e.g., lipid, gelatinized starch, zeta potential and solubility) and each functional property (e.g., WHC, OHC, FC, FS, EA, ES, solubility and RVA-viscosities), as well as for significantly different physicochemical (e.g., lipid, gelatinized starch, zeta potential, solubility, tannins, polyphenols, TIA and CIA) and *in vitro* protein digestibility. All statistical analysis was performed using the Systat 10 statistical software (Systat Software Inc., San Jose, CA, USA).

4. RESULTS AND DISCUSSION

4.1. Effect of tempering moisture and infrared heating temperature on the physicochemical and functional properties of chickpea and barley flour, and their blends

4.1.1. **Physicochemical properties**

(a) Composition

Compositional analysis (protein, ash, crude fat, total starch and gelatinized starch; reported on a dry weight basis) of flours derived from untreated and thermally treated Desi chickpeas and hull-less barley were assessed (Table 4.1.1). In the case of un-tempered non-micronized Desi chickpea flour (control), protein, ash, crude fat and total starch levels were found to be ~25.4%, 3.0%, 5.5% and 42.4%, respectively. In contrast, un-tempered non-micronized hull-less barley flour (control) had protein, ash, crude fat and total starch levels of ~11.4%, 1.9%, 2.3% and 60.0%, respectively. A one-way analysis of variance (ANOVA) indicated that the addition of infrared heat (115 and 135^{o}C) with and without tempering had no significant effect on the composition of either flours ($p>0.05$). The results of proximate composition fell within the range reported in previous studies on different varieties of chickpeas and barley. Chickpeas have been found to comprise of 24.0 to 26.2% protein, 3.1 to 3.6% ash and 4.2 to 5.0% fat, on a dry weight basis (Ma et al., 2011; Marconi et al., 2000; Milán-Carrillo et al., 2000). In contrast, barley has been reported to contain 9.5 to 13.3% protein, 1.5 to 1.6% ash, 1.7 to 2.3% fat and 49.0 to 67.0% total starch (Abraha et al., 2013; Emami et al., 2011; Fasina et al., 1999).

The gelatinized starch content in both chickpea and barley flours was significantly increased with the infrared temperature treatment and the tempering-heat combination ($p<0.05$) (Table 4.1.1). In the case of chickpea flour, the gelatinized starch concentration increased from ~4% in the un-tempered flour heated to 115^{o}C to ~16% when tempered and heated to the same temperature ($p<0.05$). No significant effect was observed when the flour was heated to 115 vs 135^{o}C ($p>0.05$) (Table 4.1.1). Similarly, the gelatinized starch in barley also significantly increased after tempering, rising from ~11% to ~14% with tempering at 115^{o}C, and from ~11% to ~40% at 135^{o}C ($p<0.05$)

Table 4.1.1 Compositional analysis of untreated and treated flours prepared from Desi chickpeas and hull-less barley, with and without tempering and infrared heating to different surface temperatures.

Treatment	Protein (%, db)	Ash (%, db)	Crude fat (%, db)	Total starch (%, db)	Gelatinized starch (%, db)
a) Desi chickpeas					
Un-tempered; non-micronized	25.4 ± 0.8^{a}	3.0 ± 0.0^{a}	5.5 ± 0.0^{b}	42.4 ± 2.2^{a}	n.d.
Un-tempered; heated to 115ºC	25.1 ± 0.8^{a}	3.1 ± 0.0^{a}	5.3 ± 0.0^{c}	44.2 ± 3.8^{a}	3.9 ± 1.0^{b}
Un-tempered; heated to 135ºC	26.2 ± 0.6^{a}	3.1 ± 0.0^{a}	5.5 ± 0.1^{b}	40.9 ± 0.3^{a}	4.0 ± 0.9^{b}
Tempered to 20% moisture; heated to 115ºC	25.9 ± 0.5^{a}	3.1 ± 0.0^{a}	5.7 ± 0.1^{a}	40.0 ± 2.3^{a}	16.1 ± 1.7^{a}
Tempered to 20% moisture; heated to 135ºC	26.6 ± 0.5^{a}	3.1 ± 0.0^{a}	5.7 ± 0.0^{a}	42.3 ± 0.9^{a}	16.8 ± 1.7^{a}
b) Hull-less barley					
Un-tempered; non-micronized	11.4 ± 0.3^{A}	1.9 ± 0.0^{A}	2.3 ± 0.0^{A}	60.0 ± 2.4^{A}	n.d.
Un-tempered; heated to 115ºC	10.7 ± 1.1^{A}	1.8 ± 0.0^{A}	2.6 ± 0.1^{A}	55.7 ± 2.0^{A}	10.7 ± 0.2^{B}
Un-tempered; heated to 135ºC	10.0 ± 1.2^{A}	1.8 ± 0.0^{A}	2.6 ± 0.1^{A}	58.2 ± 2.0^{A}	11.1 ± 0.3^{B}
Tempered to 20% moisture; heated to 115ºC	11.4 ± 0.5^{A}	1.8 ± 0.0^{A}	2.5 ± 0.4^{A}	58.4 ± 1.6^{A}	14.0 ± 0.9^{B}
Tempered to 20% moisture; heated to 135ºC	11.2 ± 0.3^{A}	1.9 ± 0.1^{A}	2.5 ± 0.1^{A}	59.2 ± 0.9^{A}	40.5± 2.5^{A}

Data represent the mean values from triplicate processing runs ± one standard deviation (n = 3). Similar letters within the same column indicate no significant difference ($p<0.05$). Data with lower case and upper case letters were compared separately. Abbreviations: n.d. denotes 'not determined'.

(Table 4.1.1). The higher percent of gelatinization in barley than in chickpeas with the tempered/135^{o}C treatment may be the result of barley having less protein. Chandrashekar and Kerlies (1988) proposed that the presence of proteins may surround the starch granules to inhibit the starch gelatinization process. Furthermore, it is proposed the higher amount of lipid within the chickpea flour may also limit the available water for gelatinization.

(b) Colour

The colour of the grains or flours can affect the colour of the end product and therefore is closely related to consumer acceptability. Heating may cause the Maillard reaction to take place which is accompanied by the formation of dark, brown pigments, which may hinder the potential utilization of the grains. Changes to the tri-stimulus colour profiles for untreated and treated Desi chickpeas and hull-less barley are given in Table 4.1.2. Although significant differences were found in all tri-stimulus parameters (L*, a* and b*) ($p<0.05$) for untreated and treated Desi chickpea flours, only a* values changed substantially. L* values were found to decrease slightly from ~87.6 for the un-tempered non-micronized Desi chickpea flour to ~85.5 for the seed which underwent the greatest processing (tempered to 20% moisture, heated to a surface temperature of 135^{o}C) but the effects of different surface temperature or the tempering moisture were not significantly related to the change of L*. In contrast, b* values ranged from 20.4 to 22.4 depending on the treatment, showing a significant influence by both surface temperature and tempering moisture. The un-tempered non-micronized Desi chickpea flour was found to have an a* value of ~1.9, which declined to 1.5 with the addition of infrared heating to a seed surface temperature of 115^{o}C, and again declined further with an increased thermal treatment to 135^{o}C (a* = ~1.1), and with tempering to a 20% moisture with the addition of temperature (115 or 135^{o}C) (a* = ~0.8). The value of a* was significantly affected by the surface temperature and the tempering moisture. The interaction of heating temperature and tempering moisture also significantly affected the redness values. Considering the strong correlation coefficient between the a* and b* in chickpeas ($r = 0.908$, $p<0.05$), it is possible that some compounds with a red/yellow colour were removed by tempering and heating.

In barley flours, the L* value for the un-tempered non-micronized sample was 83.8, similar to the treated samples which had values between 83.4 and 84.6. The lightness was significantly affected by surface temperature but not by tempering moisture, indicating a dark colour would be

Table 4.1.2 Tri-stimulus colour values and surface charge for untreated and treated flours prepared from Desi chickpeas and hull-less barley, with and without tempering and infrared heating to different surface temperatures.

Treatment	Tri-stimulus colour values			Surface charge (mV)
	L*	a*	b*	
a) Desi chickpeas				
Un-tempered; non-micronized	87.56 ± 0.48^{a}	1.89 ± 0.11^{a}	21.15 ± 0.57^{b}	-35.37 ± 1.48^{a}
Un-tempered; heated to 115°C	86.84 ± 0.20^{ab}	1.52 ± 0.17^{b}	22.44 ± 0.14^{a}	-41.45 ± 0.82^{b}
Un-tempered; heated to 135°C	86.44 ± 0.39^{ab}	1.09 ± 0.06^{c}	21.45 ± 0.27^{ab}	-40.47 ± 0.65^{b}
Tempered to 20% moisture; heated to 115°C	86.26 ± 1.24^{ab}	0.79 ± 0.05^{d}	20.77 ± 0.58^{b}	-42.63 ± 1.48^{b}
Tempered to 20% moisture; heated to 135°C	85.49 ± 0.76^{b}	0.85 ± 0.13^{cd}	20.43 ± 0.55^{b}	-41.35 ± 0.35^{b}
b) Hull-less barley				
Un-tempered; non-micronized	83.76 ± 0.34^{AB}	1.50 ± 0.01^{C}	11.04 ± 0.12^{C}	-24.17 ± 1.40^{A}
Un-tempered; heated to 115°C	83.87 ± 0.66^{AB}	1.59 ± 0.04^{B}	11.49 ± 0.10^{B}	-27.02 ± 1.57^{AB}
Un-tempered; heated to 135°C	83.45 ± 0.29^{B}	1.63 ± 0.05^{B}	11.74 ± 0.06^{A}	-28.65 ± 1.78^{B}
Tempered to 20% moisture; heated to 115°C	84.59 ± 0.42^{A}	1.46 ± 0.08^{C}	11.07 ± 0.18^{C}	-25.27 ± 1.64^{AB}
Tempered to 20% moisture; heated to 135°C	83.76 ± 0.15^{AB}	1.73 ± 0.04^{A}	11.30 ± 0.11^{B}	-23.98 ± 1.29^{A}

Data represent the mean values from triplicate processing runs ± one standard deviation (n = 3). Similar letters within the same column indicate no significant difference ($p<0.05$). Data with lower case and upper case letters were compared separately.

expected when barley flours were heated to a higher surface temperature. The a* value in barley was significantly affected by heating temperature as well as the interaction between temperature and tempering moisture. The a* value for the un-tempered non-micronized sample was 1.5. It significantly increased after heating to 115 or 135^{o}C without tempering and further increased to 1.7 when tempered and heated to 135^{o}C. Both temperature and tempering moisture had a significant effect on b* in barley but their interaction had no significant influence on the b* values. The b* value in un-tempered non-micronized barley samples was 11.0 and it significantly increased to 11.5 and 11.7 when directly heated to 115 and 135^{o}C, respectively. The barley sample tempered and heated to 115^{o}C was not significantly different from the control sample, with a value of 11.1. A significant increase to 11.3 was found when the samples were tempered and heated to 135^{o}C. The b* was also significantly ($p<0.05$) correlated with the L* ($r = -0.664$). The darkening of flour is usually associated with Maillard browning, a non-enzymatic process involving heat and the reaction between an amino acid and a reducing sugar resulting in the formation of brown pigments.

(c) Surface charge (zeta potential)

The surface charge of a protein indicates the likelihood of the protein to remain in solution via electrostatic repulsion. Higher surface charge (>30 mV or <-30 mV) exerts a strong repulsion in the colloidal system and hence dispersion stability is obtained (Wu et al., 2015). As shown in Table 4.1.2, both chickpea and barley flours were negatively charged at pH 7.0, since they are above their respective isoelectric points [chickpea pI = 4.8 (Márquez and Alonso, 1999); barley pI ~6 (Casella and Whitaker, 1990)]. The un-tempered, unheated chickpea flour carried a high charge of -35 mV, indicating that the chickpea proteins would possibly be dispersed in the solution by electrostatic repulsive means. Upon heating, with or without tempering, the surface charge became significantly greater ~-40-43 mV ($p<0.05$). The greater charge was most likely caused by the partial unravelling of the protein structure upon processing which brought a greater amount of charged amino acids to the surface. In contrast, the surface charge for barley ranged between ~-24 mV to ~-29 mV depending on the processing conditions (Table 4.1.2). Although there were slight differences within treatments, no significant trends could be discerned. The lower surface charge in barley relative to chickpea samples is most likely caused by the solution pH (7.0), which is

closer to the pI of barley than chickpeas, and the total amount of charged amino acids present (44% vs 50%). The barley protein solution would thus be less stable than that of the chickpea proteins.

4.1.2. **Functional properties**

(a) Solubility

The protein solubility of treated and untreated Desi chickpea and hull-less barley flours is shown in Table 4.1.3. A one-way ANOVA indicated a significant decrease in protein solubility with processing in both chickpea and barley flours. The highest solubility of chickpea and barley flours was for the untreated samples which were 69.5% and 27.0% soluble, respectively, whereas the lowest solubility was found in the tempered and heated (135^{o}C) flour which had a solubility of 43.5% for chickpea flour and 10.7% for barley flour. The solubility of chickpea and barley flours was significantly affected by both temperature and the tempering-heat combination ($p<0.05$) with the latter producing a greater reduction in solubility for both flours ($p<0.05$). Other studies also reported a more significant heat-induced protein solubility reduction after tempering. Pathiratne et al. (2015) reported the protein dispersibility index for lentil flour to decrease with the increase of infrared heating temperature from 115 to 165^{o}C and increasing moisture levels from 8 to 23%. Arntfield et al. (1997) reported lentil flour with higher levels of moisture during infrared heating to be more susceptible to protein denaturation and aggregation. A similar decrease in solubility after heat treatment was previously reported for cowpea with boiling [soaking with 1:6 (w/w) water for 24h], and infrared heating at 130 and 170^{o}C with 41% tempering moisture (Mwangwela et al., 2007b; Prinyawiwatkul et al., 1997) and in lentils when micronized to 138 and 170^{o}C with 31% tempering moisture (Arntfield et al., 2001). Ma et al. (2011) found that a wet heating process (boiling) induced significantly lower protein solubility than a dry heating process (roasting) in hulled and dehulled lentil flour, dehulled Desi and Kabuli chickpea flour and dehulled yellow flour.

Table 4.1.3 Functional properties of untreated and treated flours prepared from Desi chickpeas and hull-less barley, with and without tempering and infrared heating to different surface temperatures.

Treatment	Solubility (%)	OHC (g oil / g of flour d.b)	WHC (g water/g of flour d.b)	Emulsion activity (%)	Emulsion stability (%)	Foaming Capacity (%)	Foaming stability (%)
a) Desi chickpeas							
Un-tempered; non-micronized	69.52 ± 1.79^{a}	1.13 ± 0.02^{a}	1.10 ± 0.01^{d}	44.5 ± 0.5^{b}	47.4 ± 1.7^{a}	181.4 ± 4.6^{b}	85.5 ± 6.1^{ab}
Un-tempered; heated to 115°C	66.00 ± 1.65^{a}	1.04 ± 0.04^{a}	1.34 ± 0.05^{c}	44.8 ± 0.6^{ab}	49.6 ± 2.0^{a}	214.7 ± 2.1^{a}	92.8 ± 1.3^{a}
Un-tempered; heated to 135°C	55.79 ± 1.82^{b}	1.17 ± 0.08^{a}	1.59 ± 0.08^{b}	45.1 ± 0.2^{ab}	50.1 ± 0.9^{a}	186.3 ± 6.7^{b}	89.2 ± 2.3^{ab}
Tempered to 20% moisture; heated to 115°C	47.93 ± 3.00^{c}	1.18 ± 0.06^{a}	1.66 ± 0.07^{ab}	46.0 ± 1.0^{ab}	47.7 ± 0.9^{a}	131.5 ± 8.0^{c}	84.1 ± 3.5^{ab}
Tempered to 20% moisture; heated to 135°C	43.53 ± 2.00^{c}	1.06 ± 0.08^{a}	1.78 ± 0.04^{a}	47.1 ± 1.6^{a}	48.4 ± 1.4^{a}	127.5 ± 0.8^{c}	79.3 ± 5.9^{b}
b) Hull-less barley							
Un-tempered; non-micronized	27.02 ± 1.78^{A}	1.37 ± 0.02^{A}	1.50 ± 0.04^{C}	7.1 ± 0.0^{A}	17.6 ± 1.8^{A}	64.7 ± 11.3	54.5 ± 7.8
Un-tempered; heated to 115°C	16.29 ± 0.55^{B}	1.46 ± 0.12^{A}	1.62 ± 0.06^{BC}	1.8 ± 0.0^{B}	3.3 ± 0.7^{B}	n.d.	n.d.
Un-tempered; heated to 135°C	12.50 ± 0.75^{C}	1.31 ± 0.06^{A}	1.70 ± 0.12^{B}	1.5 ± 0.5^{B}	2.5 ± 0.2^{B}	n.d.	n.d.
Tempered to 20% moisture; heated to 115°C	11.76 ± 0.30^{C}	1.44 ± 0.08^{A}	1.75 ± 0.03^{B}	1.4 ± 0.1^{B}	2.0 ± 0.5^{B}	n.d.	n.d.
Tempered to 20% moisture; heated to 135°C	10.72 ± 0.36^{C}	1.48 ± 0.11^{A}	2.82 ± 0.08^{A}	2.1 ± 0.4^{B}	2.4 ± 0.8^{B}	n.d.	n.d.

Data represent the mean values from triplicate processing runs ± one standard deviation (n = 3). Similar letters within the same column indicate no significant difference ($p<0.05$). Data with lower and upper letters were compared separately. Abbreviations: n.d. denotes 'not detected'.

In the present study, the loss of solubility with processing is presumed to be associated with the exposure of hydrophobic groups as the proteins partially unravel, which would induce a greater amount of protein-protein aggregation. This effect is believed to overcome any minor increases in surface charge during the denaturation process. The greater overall solubility in the chickpea flours reflects the higher amounts of albumin- (13% of total proteins – water soluble) and globulin-type (57% of total proteins – salt soluble) proteins present (Singh and Jambunathan, 1982). In contrast, barley proteins are dominated by prolamin proteins [33 - 55% of total proteins (Helm et al., 2004)] which are more hydrophobic in nature (alcohol soluble). The higher lipid content in chickpeas (~5.7%) will act to interact with the proteins in solution to reduce their solubility ($r = -0.815$, $p<0.001$) (Table 4.1.4). Similarly, as the amount of gelatinized starch increases solubility will decrease for both chickpea ($r = -0.907$, $p<0.001$) and barley ($r = -0.692$, $p<0.01$) flour as the increased protein-amylose interactions are hypothesized to increase the rate of sedimentation of the proteins (Tables 4.1.4). In general, solubility tends to improve as the surface charge on the protein increases due to electrostatic repulsion in solution, however, in the present study the opposite relationship was found ($r = 0.601$, $p<0.05$). In this case, it was thought that the changes to protein conformation during heating, and the subsequent exposure of hydrophobic amino acids dominated over any effects of surface charge. The different factors are because of their respective composition, where chickpeas are much higher in lipid content and lower in starch than barley.

(b) Oil holding capacity

The oil holding capacities (OHC) for untreated and treated Desi chickpea and hull-less barley flours are given in Table 4.1.3. A one-way ANOVA indicated there were no significant differences between any of the treatments ($p>0.05$) where OHC for Desi chickpea and hull-less barley flours ranged between 1.0 to 1.2 g/g, and between 1.3 to 1.5 g/g, respectively. Correlation analysis indicated that none of the physicochemical properties related to OHC ($p<0.05$) for either flour (Tables 4.1.4).

Table 4.1.4. Pearson correlation (pairwise) results describing the relationships between the physicochemical and functional properties of processed Desi chickpea and hull-less flour.

Functional property	Desi chickpea	Hull-less barley
Solubility	Lipid ($r = -0.815$, $p<0.001$) GS ($r = -0.907$, $p<0.001$) ZP ($r = -0.601$, $p<0.05$)	GS ($r = -0.692$, $p<0.01$)
Water hydration capacity	Lipid ($r = 0.634$, $p<0.05$) GS ($r = 0.828$, $p<0.001$) Sol ($r = -0.947$, $p<0.001$) ZP ($r = 0.721$, $p<0.01$)	GS ($r = 0.969$, $p<0.001$) Sol ($r = -0.551$, $p<0.05$)
Oil holding capacity	Non-significant	Non-significant
Foaming capacity	Lipid ($r = -0.914$, $p<0.001$) GS ($r = -0.856$, $p<0.001$) Sol ($r = 0.833$, $p<0.001$)	Non foaming
Foaming stability	Lipid ($r = -0.650$, $p<0.01$) GS ($r = -0.594$, $p<0.05$)	Non foaming
Emulsion activity	Lipid ($r = 0.633$, $p<0.05$) GS ($r = 0.750$, $p<0.01$) Sol ($r = -0.706$, $p<0.01$)	Sol ($r = 0.931$, $p<0.001$)
Emulsion stability	Non-significant	Sol ($r = 0.955$, $p<0.001$) GS ($r = -0.575$, $p<0.05$)
Rapid visco-analyzer		
Peak viscosity (PV)	Lipid ($r = -0.714$, $p<0.01$) GS ($r = -0.868$, $p<0.001$) Sol ($r = 0.906$, $p<0.001$) ZP ($r = -0.666$, $p<0.01$)	GS ($r = -0.645$ $p<0.01$)
Trough viscosity (TV)	Lipid ($r = -0.729$, $p<0.01$) GS ($r = -0.883$, $p<0.001$) Sol ($r = 0.920$, $p<0.001$) ZP ($r = -0.655$, $p<0.01$)	GS ($r = -0.515$, $p<0.05$)
Breakdown viscosity (BV)	Lipid ($r = -0.673$, $p<0.01$) GS ($r = -0.887$, $p<0.001$) Sol ($r = 0.904$, $p<0.001$) ZP ($r = -0.708$, $p<0.01$)	GS ($r = -0.702$, $p<0.01$)
Setback viscosity (SV)	Non-significant	GS ($r = -0.655$, $p<0.01$)

N = 15, df = 13, Pearson correlation coefficients [$r = 0.514$ ($p<0.05$); $r = 0.641$ ($p<0.01$); and $r = 0.760$ ($p<0.001$)]
Abbreviations: (Sol) solubility; (GS) gelatinized starch content; (ZP) zeta potential

Ma et al. (2011) reported that roasting did not significantly change the fat absorption capacity of chickpeas. Aguilera et al. (2009) also found no significant changes of OHC in chickpeas after soaking for 20 h, followed by boiling for 70 min with or without dehydration afterwards. The higher OHC capacity within the hull-less barley is thought to reflect the increased hydrophobic nature of the proteins associated with the dominant prolamin-type protein fraction. In contrast, chickpea flours are dominant with more hydrophilic albumin and globulin-type protein classes. Ashraf et al. (2012) also found that cereal (wheat) flour had a higher oil binding capacity comparing to pulse (red bean) flour and attributed this to the lower amount of hydrophobic amino acids in the pulse flour.

(c) Water hydration capacity

The water hydration (WHC) capacities for untreated and treated Desi chickpea and hull-less barley flours are given in Table 4.1.3. A one-way ANOVA found significant differences in WHC for both flours and also treatments ($p>0.05$) (Table 4.1.3). In the case of Desi chickpea flour, WHC increased from the un-tempered non-micronized flours (WHC = 1.1 g/g) to ~1.3 g/g and ~1.6 g/g with the addition of heat to 115^{o}C and 135^{o}C, respectively. WHC values increased further to ~1.7 g/g and ~1.8 g/g with the combination of tempering to 20% moisture plus heating to 115^{o}C and 135^{o}C, respectively. A similar trend was found with hull-less barley, where the WHC increased slightly from ~1.5 g/g for un-tempered non-micronized flour to ~1.6-1.7 g/g for heat-treated seeds to 115/135^{o}C or tempered to 20% moisture and 115^{o}C infrared heat. WHC then increased further by heating tempered seeds to 135^{o}C (WHC ~2.8 g/g).

The rise in WHC values with treatment is hypothesized to be due to the following: temperature induced unraveling of the protein conformation which exposed an increased number of previously buried hydrophilic moieties to the surface; an increase in the degree of starch gelatinization [($r = 0.828$ – chickpea), ($r = 0.969$ – barley), $p<0.001$] (Table 4.1.4); and the structural changes caused by the imbibition-removal of water leaving greater porosity for water to bind (Ma et al., 2011). This change in structure is also reflected indirectly by a positive correlation with surface charge ($r = 0.721$, $p<0.01$) and a negative correlation with solubility ($r = -0.947$, $p<0.001$) in the case of chickpea flour (Table 4.1.4). Research also has reported that instead of protein solubility, the water binding capacity is more related to the protein content (Hermansson,

1979; Hutton and Campbell, 1989). Higher values of WHC seen for the hull-less barley relative to the Desi chickpeas are thought to be associated with its higher amount of starches and more gelatinized starch as well as the beta-glucan that can absorb water (Aguilera et al., 2009). Similar results were also reported by Ma et al. (2011), where they found that both roasting and tempering increased WHC in dehulled Desi from ~0.79 g/g to ~0.85 g/g after roasting and ~1.5 g/g after boiling, and by Pathiratne et al. (2015) where the water holding capacity of lentil flour increased from ~0.8 g/g to ~0.9 and 1.0 g/g when tempered to 15% moisture and infrared heated to 115 and 130^{o}C, and further increased to ~1.5 and 2.2 g/g when tempered to 23% moisture and heated to 115 and 130^{o}C separately.

(d) Emulsifying properties

Since proteins are amphiphilic in nature, they can diffuse and absorb to the oil-water interface and bridge both the water and oil phases to lower the interfacial tension (Lam and Nickerson, 2013, Ashraf et al., 2012). The emulsifying activity (EA) and stability (ES) for untreated and treated Desi chickpea and hull-less barley flours are given in Table 4.1.3. A one-way ANOVA found that the EA values increased significantly from 44% in the case of un-tempered non-micronized Desi chickpea flour to 47% for seeds tempered to 20% moisture and heated to a surface temperature of 135^{o}C ($p<0.05$). The emulsion activity for chickpea flour was positivity correlated with both the gelatinized starch content ($r = 0.750$, $p<0.001$) and lipid content ($r = 0.633$, $p<0.01$), and negatively correlated with solubility ($r = -0.706$, $p<0.01$) (Table 4.1.4). The rise in the emulsion forming properties is believed to be associated with the unravelling of the protein structure to expose previously buried hydrophobic amino acids resulting in some loss in protein solubility. During emulsion formation, proteins migrate to the oil-water interface, then realign to position its hydrophobic and hydrophilic moieties towards the oil and water phases, respectively, to lower interfacial tension. The presence of residual lipid associated with the protein would also aid in emulsification. Aggregation of the proteins then ensues at the interface to form a viscoelastic interfacial film. The increase of EA was possibly associated with the protein-carbohydrate interactions at the oil-water interface (Ma et al., 2011), caused by the increased presence of gelatinized starch. However, this did not translate into significant differences ($p>0.05$) in ES of chickpeas under different treatments, where the ES fell within the range of 47 to 50%. Emulsions are thought to be stabilized through steric forces and electrostatic repulsive forces

arising from the viscoelastic interfacial film and increased continuous phase viscosity because of the starch gelatinization (Lam and Nickerson, 2013). In the case of hull-less barley, a one-way ANOVA analysis found both EA and ES experienced a significant decline from 7% and 18%, respectively for un-tempered non-micronized flours to ~2% and ~3%, respectively for the processed seed ($p<0.05$) (Table 4.1.3). No differences were found with tempering or between infrared heating temperatures ($p>0.05$), suggesting that the addition of heat was most likely the reason for the decline relative to the control. For barley flour, EA was positively correlated with solubility ($r = 0.931$, $p<0.001$) (Table 4.1.4). EA was improved with increased solubility in the case of barley since soluble proteins were needed to migrate to the interface. This was opposite to chickpea, where EA declined with increased solubility possibly since fewer proteins remained in solution rather than interacting with the interface. ES was found to be correlated positively with solubility ($r = 0.955$, $p<0.001$) and negatively with gelatinized starch content ($r = -0.575$, $p<0.05$) (Table 4.1.4). Similar to EA, having some protein in solution is important to stabilize the interface and to increase the viscosity of the continuous phase. Having higher amounts of gelatinized starch would also reduce emulsion viscosity leading to faster gravitational separation.

(e) Foaming properties

The foaming capacity (FC) and stability (FS) for untreated and treated Desi chickpea and hull-less barley flours are given in Table 4.1.3. A one-way ANOVA found significant differences for chickpea flour among the treatments for both FC ($p<0.05$) and FS ($p<0.05$). Un-tempered non-micronized Desi chickpea flours had a FC value of ~181%, which increased to ~217% when heated to a surface temperature of 115^{o}C, then declined to ~186% when heated to 135^{o}C. When seeds were tempered to 20% moisture and heated (115/135^{o}C), FC declined further to ~130%. FC was found to be positively correlated with solubility ($r = 0.833$, $p<0.001$) and negatively correlated with lipid ($r = -0.914$, $p<0.001$) and gelatinized starch content ($r = -0.856$, $p<0.001$) (Table 4.1.4). Proteins contribute to foam formation by migrating to the air-water interface, unravelling and re-aligning similar to emulsions where hydrophobic and hydrophilic moieties are positioned towards the air and water phases, respectively. If solubility is greater, then more proteins will migrate to the interface. Once at the interface, viscoelastic interfacial films form. It was hypothesized that the initial rise in FC when seeds were heated to 115^{o}C was attributed to the partial unfolding of the protein structure, where if too much denaturation occurred (with higher processing), the proteins

would have issues migrating to the air-water interface. It is also hypothesized that the higher amounts of the gelatinized starch present will lead to reduced viscosity within the foaming solution which will reduce the amount of foam being produced and ultimately its stability.

In the case of FS, a similar trend to FC was evident. Un-tempered non-micronized Desi chickpea flours had a FS of ~85%, which increased to a FS of ~93% with the addition of heat (115^{o}C). FS subsequently declined when heated to 135^{o}C (FS ~89%), or with tempering to 20% moisture and heating to 115^{o}C (FS ~84%) or 135^{o}C (FS ~79%). FS was found to be negatively correlated with lipid content ($r = -0.650$, $p<0.01$) and gelatinized starch content ($r = -0.594$, $p<0.05$) (Table 4.1.4). The significant improvement in un-tempered 115^{o}C-heated chickpea samples correlated with the significant increase in absolute zeta potential, with a solubility close to the untreated sample. Further, the amount of gelatinized starch is much less than in those which have undergone higher levels of processing meaning the viscosity of the foaming solution between the air bubbles would be greater, leading to the enhanced stability. The foaming results decreased in the rest of the chickpea samples, which is possibly due to the limit in soluble protein in the solution.

In the case of hull-less barley flours, FC and FS for the un-tempered non-micronized flour were found to be ~65% and 54%, respectively (Table 4.1.3). Foaming was prevented with the addition of heat or tempering + heating. The lack of foam formation indicates that the proteins were unable to stabilize the air-water interface. Although the surface charge improved in barley samples to some extent, only the samples heated to 135^{o}C without tempering showed a significant increase in the absolute value, indicating the improvement of surface charge could not compensate for the loss of soluble proteins.

(f) Pasting properties

The pasting curves of treated and untreated chickpea and barley flours are shown in Figure 4.1.1, with corresponding parameters given in Table 4.1.5. These include, a) peak viscosity *(i.e., the maximum viscosity during the heating and holding stage)*; b) trough viscosity *(i.e., the minimum viscosity after the peak viscosity)*; c) breakdown *(i.e., peak minus trough viscosity)*; d) setback *(i.e., final viscosity minus trough viscosity);* and e) pasting temperature *(i.e., the temperature required to form a paste)*. In the case of Desi chickpea flour suspensions, peak, trough and breakdown viscosities were significantly lower with increasing infrared heating temperature (un-tempered) ($p<0.05$), an effect which was magnified when the seeds were tempered first

($p<0.05$) (Table 4.1.5). The setback viscosity was more variable among treatments. Pearson correlation (Table 4.1.4) found that the solubility was positively correlated with peak viscosity ($r = 0.906$, $p<0.001$), trough viscosity ($r = 0.920$, $p<0.001$) and breakdown viscosity ($r = 0.904$, $p<0.001$), but these viscosities were negatively correlated with lipid (peak viscosity, $r = -0.714$, $p<0.01$; trough viscosity, $r = -0.729$, $p<0.001$; breakdown viscosity, $r = -0.673$, $p<0.01$), gelatinized starch content (peak viscosity, $r = -0.868$, $p<0.001$; trough viscosity, $r = -0.883$, $p<0.001$; breakdown viscosity, $r = -0.887$, $p<0.001$) and zeta potential (peak viscosity, $r = -0.666$, $p<0.01$; trough viscosity, $r = -0.655$, $p<0.01$; breakdown viscosity, $r = -0.708$, $p<0.01$), despite none of these physicochemical properties correlating with setback viscosity ($p>0.05$). In contrast, in the barley samples, only the gelatinized starch content was found correlated with peak ($r = -0.645$, $p<0.001$), trough ($r = -0.515$, $p<0.05$), breakdown ($r = -0.702$, $p<0.01$) and setback viscosity ($r = -0.655$, $p<0.01$). However, the magnitude of each viscosity was found to be much greater than the chickpea flour suspensions.

The increase of viscosity before peak viscosity is caused by the swelling of starch granules and the leaching of amylose from the organized starch structure into the external matrix (Crosbie and Ross, 2007; Kaur et al., 2015). Heating and tempering during the sample preparation induced starch gelatinization and granule swelling so there were fewer granules which swelled during the pasting process, leading to a decline in peak viscosity (Wani et al., 2016). A similar decline was reported by Emami et al. (2010b) on barley flour that was tempered (42-45% moisture content) and infrared heated to 100^{o}C and by Al-Rabadi et al. (2011) for pre-heated barley and sorghum extrudates. The decrease of viscosity following the peak is caused by the breakdown of granules. Most of the pulse starches tend to have restricted swelling and less amylose leaching possibly due to strong interactions between starch chains and the formation of strong aggregates with proteins or other components (Hoover et al., 2010; Sharma and Kotari, 2017), leading to a low or even an absence of peak viscosity during the pasting test. This phenomenon was found in the chickpea flour suspensions in the present study, where the peak was lost when the samples were heated after tempering and a breakdown viscosity of zero was found. Relative to other pulse flours (e.g., pinto beans, lima beans, mung beans, lentils etc.), chickpea flour shows lower viscosity values which are thought to be caused by fewer interactions with water due to the high content of lipid present (Du et al., 2014). Upon cooling, the retrogradation occurs as amylose polymers start to reconnect with each other outside of the granule leading to a substantial increase in viscosity as the

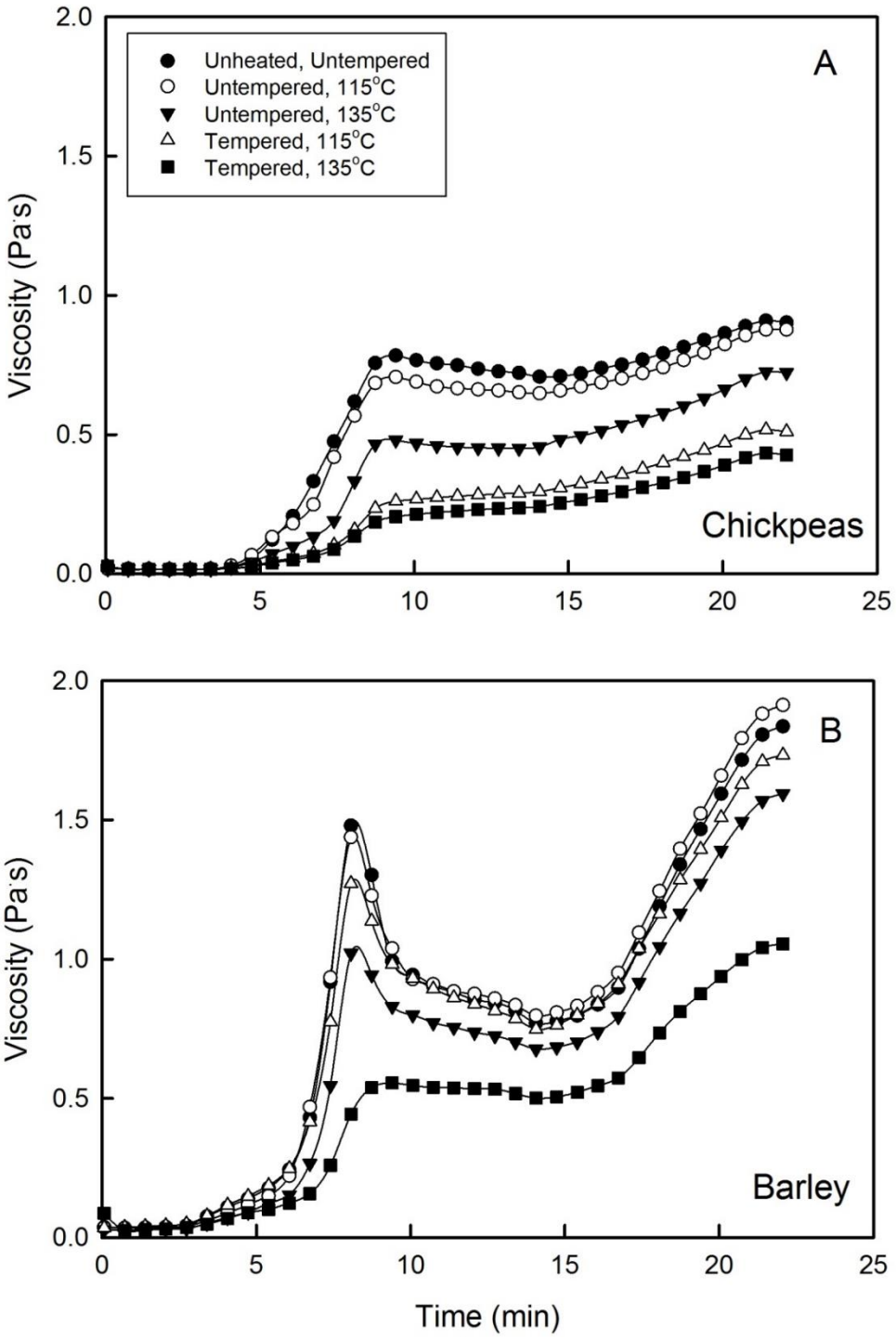

Figure. 4.1.1 Pasting curves of treated and untreated (a) Desi chickpea and (b) hull-less barley flours.

Table 4.1.5 Pasting properties of untreated and treated flours prepared from Desi chickpeas and hull-less barley, with and without tempering and infrared heating to different surface temperatures.

Treatment	Peak viscosity (Pa·s)	Trough (Pa·s)	Breakdown (Pa·s)	Setback (Pa·s)	Pasting temperature (°C)
a) Desi chickpeas					
Un-tempered; non-micronized	0.79 ± 0.19^{a}	0.70 ± 0.15^{a}	0.08 ± 0.03^{a}	0.19 ± 0.05^{ab}	73.8 ± 2.4^{c}
Un-tempered; heated to 115°C	0.71 ± 0.04^{ab}	0.65 ± 0.03^{ab}	0.06 ± 0.01^{ab}	0.22 ± 0.01^{ab}	73.9 ± 0.7^{c}
Un-tempered; heated to 135°C	0.48 ± 0.05^{bc}	0.45 ± 0.05^{bc}	0.04 ± 0.00^{bc}	0.27 ± 0.07^{a}	88.0 ± 1.7^{b}
Tempered to 20% moisture; heated to 115°C	0.29 ± 0.02^{cd}	0.29 ± 0.02^{cd}	0.00 ± 0.00^{c}	0.21 ± 0.02^{ab}	92.4 ± 0.5^{a}
Tempered to 20% moisture; heated to 135°C	0.24 ± 0.03^{d}	0.24 ± 0.03^{d}	0.00 ± 0.00^{c}	0.18 ± 0.01^{b}	93.0 ± 0.8^{a}
b) Hull-less barley					
Un-tempered; non-micronized	1.55 ± 0.43^{A}	0.76 ± 0.12^{A}	0.79 ± 0.32^{A}	1.08 ± 0.17^{A}	78.6 ± 3.1^{A}
Un-tempered; heated to 115°C	1.52 ± 0.32^{A}	0.79 ± 0.15^{A}	0.73 ± 0.17^{AB}	1.12 ± 0.18^{A}	77.4 ± 3.3^{A}
Un-tempered; heated to 135°C	1.09 ± 0.06^{A}	0.67 ± 0.03^{A}	0.42 ± 0.03^{AB}	1.08 ± 0.26^{A}	81.3 ± 1.3^{A}
Tempered to 20% moisture; heated to 115°C	1.33 ± 0.80^{A}	0.74 ± 0.36^{A}	0.59 ± 0.44^{AB}	1.00 ± 0.47^{A}	77.1 ± 10.9^{A}
Tempered to 20% moisture; heated to 135°C	0.58 ± 0.03^{A}	0.50 ± 0.04^{A}	0.08 ± 0.01^{B}	0.56 ± 0.02^{A}	86.4 ± 0.7^{A}

Data represent the mean values from triplicate processing runs ± one standard deviation (n = 3). Similar letters within the same column indicate no significant difference ($p>0.05$). Data with lower case and upper case letters were compared separately.

suspension is cooled down (Sharma and Kotari, 2017). Setback viscosity within the RVA test gives a measurement of the re-association tendency of starch during cooling (Kaur and Singh, 2005). Chickpeas showed much lower setback viscosity than barley, indicating a resistance to starch retrogradation. The pasting properties of barley flour in the present study were less susceptible to heat and moisture than the chickpea flour. Only the barley flour tempered to 20% moisture and heated to 135^{o}C had a significantly different result, showing an absence of peak and a much lower final viscosity in the pasting curve (Figure 4.1.1). The significant difference may be associated with the significant increase of gelatinized starch content (Table 4.1.1). A sharp peak was found in barley samples, indicating that the starch granules absorbed water and swelled fast (Figure 4.1.1).The sharp decrease after the peak is possibly due to the disruption of the starch granules by shear and fewer proteins in the system, indicating that the integrated starch during heating was also rather susceptible to breakdown. The higher setback of barley samples as compared to chickpea samples was possibly due to higher starch content in barley with more available amylose-amylose and starch-protein interactions.

The decrease in viscosity, in general, indicates that the product becomes easier to cook (Bashir and Aggarwal, 2016). Bashir and Aggarwal (2016) reported that the peak viscosity usually associates with water binding capacity of the flours. In the present study, both the WHC and peak viscosity of chickpeas was lower than those of barley (Table 4.1.3 and Table 4.1.5). Breakdown viscosity is also associated with the shear thinning properties (Bashir and Aggarwal, 2016). According to the results in Table 4.1.5, the breakdown viscosity of both chickpea and barley flour decreased after heating but the setback viscosity did not change substantially. The change of viscosity of chickpea flour subjected to heating with moisture was much less extensive than barley, indicating chickpea flours might be a better ingredient for high-temperature processed food (Chung et al., 2008).

Pasting temperatures indicate the temperature needed for the starch to cook. In the present study, pasting temperatures for unheated, un-tempered chickpea flour was ~74^{o}C, which then increased to 88^{o}C as temperatures were raised to 135^{o}C (without tempering) ($p<0.05$). The addition of a tempering treatment prior to heating increased the pasting temperature to 93^{o}C for seeds heated to both 115^{o}C and 135^{o}C (Table 4.1.5). Hoover and Ratnayake (2002), and Kaur and Singh (2005) both reported the pasting temperatures of untreated chickpea flour to range between 73-75^{o}C depending on the variety of Desi chickpeas. In the case of barley, pasting temperatures were found

to be similar among all treatments, ranging between 77 and 86^{o}C (Table 4.1.5) ($p>0.05$). Native barley flour is known to have a wide range of pasting temperatures depending on the varieties tested. Zhou et al. (2008) measured the pasting temperatures of 12 different varieties and found it to range between 79 and 88^{o}C, whereas in a study by Chang and Lv (2017) a pasting temperature of 70^{o}C was reported for hull-less barley. Pasting temperatures indicate the relative ease at which the starch granules swell during cooking.

4.1.3. **Properties of blended chickpea: barley flours**

Chickpea and barley flours tempered to 20% moisture and 135^{o}C infrared heating temperature were then mixed on a weight-by-weight basis at different ratios. The proximate composition of blended flours is shown in Table 4.1.6. Protein, ash and crude fat were lowest for 100% barley flour at 11.2%, 1.9% and 2.5%, respectively, and highest for 100% chickpea flour at 26.6%, 3.1% and 5.7%, respectively. At ratios in-between, a significant increase in each parameter occurred as more and more chickpea flour replaced the barley component ($p<0.05$) (Table 4.1.6). A similar trend was seen in the surface charge data, where the lowest charge was found for 100% barley flour at -24 mV and the highest was for 100% chickpea flour at -41 mV at pH 7.0 (Table 4.1.6). Overall, blending of chickpea and barley flours resulted in a gradient change in the magnitude of the functional attribute (e.g., solubility, OHC and WHC) as the contribution of chickpea flour increased within the ratio from 0:100 to 100:0 chickpea: barley (Table 4.1.7). A One-way ANOVA found significant differences among the various blending ratios ($p<0.05$). As the concentration of chickpea flour increased from 0:100 to 100:0 chickpea: barley, protein solubility increased from ~11 to ~44%, OHC decreased from ~1.5 g/g to ~1.1 g/g, and WHC decreased from ~2.8 g/g to ~1.8 g/g (Table 4.1.7). The changes in these functional properties are hypothesized to be due to an increased concentration of more hydrophilic (water soluble) albumin and globulin proteins, and a decreased amount of more hydrophobic prolamin-type proteins. The lower WHC values with higher amounts of chickpea, despite having more protein present may be the result of a significant increase in crude fat levels which would adversely affect the trapping of water within the protein's conformation. In terms of EA and ES, values were similar when a high amount of barley present (0:100 and 20:80 chickpea: barley), then increased over the range of 40:60 and 60:40, before becoming constant as the amount of chickpea flour increased further ($p<0.05$) (Table 4.1.7). Overall, the emulsifying properties of the blends were much better when

chickpea was dominant within the ratio rather than barley. For foaming, blends where barley dominated in the ratio showed poor foaming properties. However, foaming properties became much better once chickpea flour dominated within the ratio (Table 4.1.7).

Table 4.1.6 Proximate composition and surface charge for mixed Desi chickpea and hull-less barley flours blended at different ratios on a dry weight basis. Each flour was tempered to 20% moisture followed by infrared heating to a surface temperature of 135°C.

Blending ratio	Protein (%, db)	Ash (%, db)	Crude fat (%, db)	Zeta potential (mV)
Chickpea: Barley = 0: 100	11.2 ± 0.3^{F}	1.9 ± 0.1^{F}	2.5 ± 0.2^{E}	-23.98 ± 1.29^{A}
Chickpea: Barley = 20: 80	15.0 ± 1.2^{E}	2.1 ± 0.0^{E}	3.3 ± 0.0^{D}	-24.00 ± 1.11^{A}
Chickpea: Barley = 40: 60	18.9 ± 0.7^{D}	2.3 ± 0.0^{D}	4.1 ± 0.3^{C}	-27.30 ± 1.61^{AB}
Chickpea: Barley = 60: 40	21.4 ± 0.3^{C}	2.5 ± 0.0^{C}	4.6 ± 0.0^{B}	-29.73 ± 1.46^{B}
Chickpea: Barley = 80: 20	24.4 ± 0.2^{B}	2.8 ± 0.0^{B}	5.4 ± 0.1^{A}	-34.87 ± 1.08^{C}
Chickpea: Barley = 100: 0	26.6 ± 0.5^{A}	3.1 ± 0.0^{A}	5.7 ± 0.0^{A}	-41.35 ± 0.35^{D}

Data represent the mean values from three measurements on the same blended sample ± one standard deviation (n = 3). Similar letters within the same column indicate no significant difference ($p>0.05$).

Table 4.1.7 Functional attributes of mixed Desi chickpea and hull-less barley flours at different blending ratios. Each flour was tempered to 20% moisture followed by infrared heating to a surface temperature of 135°C.

Chickpea: barley ratio	Solubility (%)	OHC (g oil / g of flour) d.b)	WHC (g water/g of flour) d.b)	Emulsion activity (%)	Emulsion stability (%)	Foaming Capacity (%)	Foaming stability (%)
0: 100	10.72 ± 0.36^{F}	1.48 ± 0.11^{A}	2.82 ± 0.08^{A}	2.14 ± 3.85^{C}	2.40 ± 0.60^{D}	n.d.	n.d.
20: 80	13.80 ± 0.51^{E}	1.46 ± 0.10^{B}	2.23 ± 0.02^{B}	4.90 ± 1.48^{C}	6.41 ± 1.67^{D}	n.d.	n.d.
40: 60	17.00 ± 0.22^{D}	1.34 ± 0.01^{B}	2.15 ± 0.08^{B}	19.78 ± 0.99^{B}	14.34 ± 4.90^{C}	35.56 ± 3.85^{C}	n.d.
60: 40	21.51 ± 1.60^{C}	1.23 ± 0.02^{C}	1.98 ± 0.02^{C}	44.41 ± 0.82^{A}	40.69 ± 3.56^{B}	97.78 ± 5.09^{B}	29.22 ± 2.67^{C}
80: 20	32.41 ± 0.21^{B}	1.24 ± 0.05^{CD}	1.85 ± 0.03^{CD}	46.97 ± 1.63^{A}	48.35 ± 2.90^{A}	126.22 ± 6.71^{A}	65.14 ± 4.24^{B}
100: 0	43.53 ± 2.00^{A}	1.06 ± 0.08^{D}	1.78 ± 0.04^{CD}	47.09 ± 1.64^{A}	48.38 ± 1.36^{A}	127.50 ± 0.83^{A}	79.25 ± 5.88^{A}

Data represent the mean values from three measurements on the same blended sample ± one standard deviation (n = 3). Similar letters within the same column indicate no significant difference ($p>0.05$).

4.2. Effect of tempering moisture and infrared heating temperature on nutritional properties of chickpea and barley flour, and their blends

4.2.1. **Anti-nutritional properties**

The presence of anti-nutritional compounds can have an adverse effect on protein digestibility and mineral absorption. Trypsin and chymotrypsin are endopeptidases that hydrolyze proteins at different sites. Trypsin and chymotrypsin inhibitors in plants are low molecular weight proteins that are used to protect seeds against bacteria and dormancy (Guillamón et al., 2008). Trypsin or chymotrypsin inhibitors can bind to lysine and arginine residues in trypsin and hydrophobic residues in chymotrypsin, respectively, to reduce the hydrolytic capacity of those enzymes (Dantzger et al., 2015). Phenolic compounds are able to cross-link proteins to inhibit their unfolding, making proteins less soluble and less susceptible to proteolytic digestion (Sreerama et al., 2012; Vidal-Valverde et al, 1994). Tannins belong to the phenol family and they can easily bind to proline and histidine (Boye et al., 2012), leading to a decrease in protein digestibility.

4.2.2. **Total phenolic and condensed tannins content**

The concentration of total phenolic content (TPC) of chickpea and barley was determined and the results were shown in Table 4.2.1. Results from the one-way ANOVA provide evidence that levels of total phenolics within Desi chickpea and barley flours significantly decreased after treatment ($p<0.05$). The total phenolics declined slightly from 1.3 mg catechin equivalent (CE)/ g of flour (d.b.), for the un-tempered non-micronized chickpea sample to 1.2 mg catechin equivalent/ g of flour for the un-tempered, heated to 115^{o}C chickpea sample. A significant reduction was found in other treated chickpea samples including the un-tempered sample heated to 135^{o}C, and the tempered samples heated to 115 or 135^{o}C ($p<0.05$). The lowest TPC of chickpea samples was found in the chickpea samples that were tempered and then heated to 135^{o}C, reaching a reduction of ~16%. The TPC of barley was higher than that of chickpea samples. The TPC was found to significantly decreased from 2.1 mg CE/g of flour for the untreated barley sample to ~ 1.7 CE/ g of flour in the treated barley flours ($p<0.05$) with the highest reduction of ~23%. Different treatments did not show a significant difference. The reduction of TPC has also been found in studies using infrared heating or other heat treatments. Xu and Chang (2008) found that boiling

Table 4.2.1 The concentration of anti-nutritional compounds in untreated and treated flours prepared from Desi chickpeas and hull-less barley, with and without tempering and infrared heating to different surface temperatures.

Treatment	**Total phenolics** (mg catechin equivalent/ g of flour, d.b.)	**Condensed tannins** (mg catechin equivalent/ g flour, d.b.)	**Trypsin Inhibitor Activity** (TIU/ mg of flour, d.b.)	**Chymotrypsin Inhibitor Activity** (CIU/ mg of flour, d.b.)
a) Desi chickpeas				
Un-tempered; non-micronized	1.28 ± 0.02^{a}	0.16 ± 0.08^{a}	16.35 ± 1.90^{a}	11.21 ± 0.37^{a}
Un-tempered; heated to 115°C	1.18 ± 0.06^{ab}	0.08 ± 0.08^{a}	14.91 ± 1.37^{a}	7.55 ± 1.15^{b}
Un-tempered; heated to 135°C	1.08 ± 0.08^{b}	0.08 ± 0.06^{a}	9.85 ± 0.49^{b}	4.38 ± 0.51^{c}
Tempered to 20% moisture; heated to 115°C	1.08 ± 1.07^{b}	0.09 ± 0.16^{a}	3.73 ± 0.55^{c}	2.18 ± 0.16^{d}
Tempered to 20% moisture; heated to 135°C	1.07 ± 0.06^{b}	0.09 ± 0.15^{a}	2.74 ± 0.53^{c}	1.55 ± 0.16^{d}
b) Hull-less barley				
Un-tempered; non-micronized	2.09 ± 0.07^{A}	1.99 ± 0.44^{A}	1.25 ± 0.28^{A}	0.50 ± 0.09^{A}
Un-tempered; heated to 115°C	1.69 ± 0.06^{B}	1.07 ± 0.04^{B}	0.80 ± 0.11^{B}	0.46 ± 0.05^{A}
Un-tempered; heated to 135°C	1.61 ± 0.05^{B}	1.05 ± 0.10^{B}	0.64 ± 0.09^{B}	0.28 ± 0.02^{B}
Tempered to 20% moisture; heated to 115°C	1.79 ± 0.08^{B}	0.76 ± 0.19^{B}	0.74 ± 0.08^{B}	0.29 ± 0.02^{B}
Tempered to 20% moisture; heated to 135°C	1.61 ± 0.10^{B}	0.85 ± 0.13^{B}	0.52 ± 0.06^{B}	0.16 ± 0.04^{B}

Data represent the mean values from triplicate processing runs ± one standard deviation (n = 3). Similar letters within the same column indicate no significant difference ($p>0.05$). Data with lower case and upper case letters were compared separately.

and steaming significantly reduced the total phenolic content in whole black soybeans. Žilić et al. (2013) found that infrared heating at 110 to 140^{o}C significantly decreased up to 98% of the TPC in maize flour. The TPC in barley decreased after extrusion at 150 or 180^{o}C with a moisture of 15% or 20% (Sharma et al., 2012). The decrease of total phenolic content could be attributed to the breakdown of phenolic compounds (Xu and Chang, 2008). However, there have been studies reporting an increase in TPC in peanut hulls under far-infrared heating, chickpeas under microwave roasting, infrared heating in soybeans etc. (Jogihalli et al., 2017; Lee et al., 2006; Žilić et al., 2014), where they attributed the increase to the breakdown of cellular compounds and the liberation of bounded phenolics. The different reaction of TPC to heat treatments are possibly associated with the variety considering the TPC in black, red and pinto dry beans showed an increase, decrease and no significant difference respectively after infrared treatment (Oomah et al., 2014). Sogi et al. (2012) also mentioned that storage time affected the susceptibility of phenolics to infrared treatment.

The levels of condensed tannins were determined, and the results are shown in Table 4.2.1. Although the content of tannins was reduced by half after treatment, only barley samples were observed to have a significant reduction in the content of condensed tannins ($p<0.05$). The levels of condensed tannins were significantly decreased from 2.0 mg CE/ g of flour for the untreated barley samples to ~1.1 mg CE/ g of flour for the heated barley samples without tempering and to ~0.8 mg CE/ g of flour for the barley samples tempered before heating ($p<0.05$). The content of tannins in untreated chickpeas ranged from traces to ~5 mg/g in various studies. Price et al. (1980) reported zero tannins in ten varieties of chickpea from five different countries and Singh (1988) also reported no detectable levels of tannins in chickpeas. Similarly, Wang et al. (2010) reported low tannin content in chickpea, with 0.04 and 0.21 CE mg/g in Kabuli and Desi chickpea, respectively, and Sharma et al. (2013) found that the tannins in Desi chickpea ranged from 1.8 to 2.2% and that in Kabuli chickpea between 0.7 to 1.3%. And Xu et al. (2016) reported 0.8 mg of gallic acid equivalent/g in Kabuli chickpea. Alajaji and El-Adawy (2006) reported the content of untreated chickpeas was 4.9 mg/g. The low levels of tannins in the chickpea could be associated with breeding practices, which focused on reducing the levels of this anti-nutritional factor. Previous studies have shown that dehulling is an effective means to remove tannins. Sudha et al. (1995) found a 65% decrease in tannins in horsegram after dehulling. Therefore, the low tannin content could result from dehulling as well. The decline in total phenolic compounds and

condensed tannins suggested that they are heat labile. Infrared heating is an effective way to reduce the levels of those compounds.

4.2.3. **Trypsin and chymotrypsin inhibitor activity**

The trypsin inhibitor activity within untreated and treated Desi chickpea and hull-less barley flour is given in Table 4.2.1. In the case of Desi chickpea flour, trypsin inhibitor activity declined from ~16.3 TIU/ mg of flour (d.b.) for un-tempered non-micronized seeds, to 14.9 and 9.3 TIU/ mg of flour (d.b.) for seeds brought to a surface temperature of 115^{o}C and 135^{o}C, respectively ($p<0.05$). The addition of 20% tempering plus heating to either a surface temperature of 115^{o}C and 135^{o}C resulted in a further decline in trypsin inhibitor activity to ~3.7 and ~2.7 TIU/ mg of flour (d.b.) ($p<0.05$), respectively. Tempering and infrared heating have both been shown to be effective in reducing trypsin inhibitor activity in various previous studies (Al-Bakir et al. 1982; Khattab and Arntfield, 2009; Márquez and Alonso, 1999). Khattab and Arntfield (2009) also reported an 89 to 94% decrease in trypsin inhibitor activity in different varieties of cowpeas, kidney beans and peas after infrared heating, which was much more effective than other methods of physical treatment including boiling, roasting, microwave cooking and autoclaving. Al-Bakir et al. (1982) reported that soaking for 24 h alone was effective in reducing trypsin inhibitor activity in chickpeas and cooking at 121^{o}C for 30 min after soaking was able to completely inactivate trypsin inhibitor activity. They also showed in the same study that trypsin inhibitor in chickpea was heat-labile and was susceptible to longer heat treatment. In the present study, levels in hull-less barley declined from ~1.2 TIU/ mg of flour (d.b.) for un-tempered non-micronized seeds to levels ranging between 0.5 and 0.8 TIU/ mg of flour (d.b.) depending on the processing conditions ($p<0.05$). However, no statistical differences were evident among the various processing treatments ($p>0.05$).

The chymotrypsin inhibitor activity showed a much similar trend as trypsin inhibitor activity (Table 4.2.1). In Desi chickpea flour, the chymotrypsin inhibitor decreased significantly from 11.2 to 7.5 CIU per mg of flour and 4.38 per mg of flour in 115^{o}C and 135^{o}C micronized flours, respectively ($p<0.05$), with a further decrease to 2.18 and 1.55 with the addition of tempering ($p<0.05$). In contrast to Desi chickpea flour, barley flour showed low chymotrypsin inhibitor activity of 0.50 unit in the un-tempered and non-micronized flour and both infrared heating and tempering showed the ability to decrease chymotrypsin inhibitor activity. The chymotrypsin inhibitor activity in barley flour decreased to 0.46 in the 115^{o}C-heated barley sample and a

significant decrease in 0.28 units per mg of flour in the 135^{o}C-heated barley sample. In the tempered barley flours, both 115 and 135^{o}C micronized samples showed a significant decline in chymotrypsin inhibitor activity with 0.29 and 0.16 units per mg of flour respectively ($p<0.05$). The trypsin and chymotrypsin inhibitors are low molecular weight proteins and tend to be heat-labile. The increase in temperature can lead to an unravelling of protein structure and a loss of biological function. The more decrease in tempered flours is thought to be due to the soluble nature of inhibitors. The dissolved proteolytic enzyme inhibitors can be removed with the removal of water (Vidal-Valverde et al., 1994).

4.2.4. **Protein quality of Desi chickpea and barley flours**

The amino acid composition (g/100 g of flour as is) for untreated and treated Desi chickpea and hull-less barley flour is given Table 4.2.2. Overall for both flour types, the amino acid levels were relatively constant regardless of whether the heat or tempering + heat was applied. The concentration of essential amino acids is given in Table 4.2.3 for untreated and treated Desi chickpea and hull-less barley flour, along with the 1990 FAO/WHO reference pattern for children 2 - 5 years of age. In the case of hull-less barley flours, threonine and lysine concentrations were lower than the reference pattern. The low lysine content was expected since it is well known that cereals are deficient in this important amino acid. Concentrations of valine, the sulphur amino acids (methionine and cysteine), isoleucine, leucine, phenylalanine and tyrosine, histidine and tryptophan were all higher than the reference pattern. For Desi chickpea, the concentration of threonine was found to be the lowest compared to the reference pattern, followed by tryptophan and sulphur amino acids (methionine and cysteine); whereas the concentration of valine, isoleucine, leucine, phenylalanine and tyrosine, histidine and lysine were greater than the required amounts. Typically, it is well known that pulses are deficient in sulphur-containing amino acids, which is interesting since the present results contradict this. The reason could be the decrease of threonine or the increase of sulphur-containing amino acid.

There are a few possible reasons for the deficient threonine content in the Desi chickpeas. [a] In Canada, pulses are typically grown in rotation with canola crops, which often require the

Table 4.2.2 Amino acid composition (g per 100 g of flour, as is basis) of untreated and treated Desi chickpea and hull-less barley flours, with and without tempering and infrared heating to different surface temperatures. Treatments include: (A) un-tempered, non-micronized; (B) un-tempered, heated to 115ºC; (C) un-tempered, heated to 135ºC; (D) tempered to 20% moisture, heated to 115ºC; and (E) tempered to 20% moisture, heated to 135ºC. Measurements were made on one processing runs only.

	ASP	GLU	SER	GLY	HIS	ARG	THR	ALA	PRO	TYR	VAL	MET	CYS	ILE	LEU	PHE	LYS	TRP
Desi chickpeas																		
A	3.01	4.45	1.56	0.99	0.80	2.51	0.74	1.01	1.06	0.69	1.03	0.30	0.33	1.04	1.89	1.44	1.77	0.26
B	3.06	4.53	1.57	1.00	0.81	2.57	0.75	1.04	1.09	0.70	1.04	0.30	0.34	1.05	1.93	1.47	1.79	0.27
C	2.98	4.43	1.54	0.98	0.82	2.53	0.74	1.02	1.07	0.68	1.01	0.31	0.33	1.02	1.88	1.43	1.74	0.27
D	3.03	4.53	1.54	0.97	0.83	2.53	0.72	1.01	1.07	0.69	1.02	0.31	0.33	1.03	1.88	1.46	1.76	0.26
E	3.20	4.74	1.64	1.02	0.88	2.67	0.78	1.04	1.09	0.72	1.04	0.31	0.33	1.04	1.90	1.44	1.76	0.27
Hull-less barley																		
A	0.69	2.65	0.53	0.43	0.28	0.54	0.30	0.45	1.22	0.26	0.51	0.13	0.20	0.36	0.74	0.54	0.39	0.13
B	0.75	2.65	0.54	0.47	0.27	0.68	0.31	0.48	1.20	0.33	0.53	0.15	0.23	0.36	0.75	0.54	0.44	0.18
C	0.73	2.63	0.54	0.46	0.26	0.67	0.31	0.47	1.20	0.32	0.52	0.15	0.22	0.35	0.74	0.53	0.42	0.18
D	0.74	2.69	0.55	0.47	0.27	0.66	0.32	0.47	1.22	0.32	0.54	0.14	0.22	0.36	0.75	0.55	0.43	0.18
E	0.75	2.73	0.57	0.48	0.27	0.67	0.32	0.49	1.25	0.33	0.54	0.15	0.22	0.36	0.76	0.55	0.43	0.18

Abbreviations: ASP, aspartate; THR, threonine; SER, serine; GLU, glutamate; PRO, proline; GLY, glycine; ALA, alanine; CYS, cysteine; VAL, valine; MET, methionine; ILE, isoleucine; LEU, leucine; TYR, tyrosine; PHE, phenylalanine; HIS, histidine; LYS, lysine; ARG, arginine; and TRP, tryptophan.

Table 4.2.3 Essential amino acid concentration (mg/g protein) of untreated and treated flours prepared from Desi chickpeas and hull-less barley, with and without tempering and infrared heating to different surface temperatures.

Treatment	THR	VAL	M+C[1]	ILE	LEU	P+T[2]	HIS	LYS	TRP
a) Desi chickpeas									
Un-tempered; non-micronized	30	41	25	42	76	86	32	71	10
Un-tempered; heated to 115ºC	30	41	25	42	76	86	32	71	11
Un-tempered; heated to 135ºC	30	41	26	41	76	85	33	70	11
Tempered to 20% moisture; heated to 115ºC	29	41	26	41	75	86	33	70	10
Tempered to 20% moisture; heated to 135ºC	30	40	25	40	73	83	34	68	10
b) Hull-less barley									
Un-tempered; non-micronized	27	47	30	33	68	73	26	36	12
Un-tempered; heated to 115ºC	29	49	35	33	69	81	25	41	17
Un-tempered; heated to 135ºC	29	49	35	33	69	79	24	39	17
Tempered to 20% moisture; heated to 115ºC	29	50	33	33	69	80	25	40	17
Tempered to 20% moisture; heated to 135ºC	29	49	33	33	69	80	24	39	16
1990 FAO/WHO reference pattern	34	35	25	28	66	63	19	58	11

Data represent the mean value of one processing run.

[1]Methionine+Cysteine; [2]Phenylalanine + Tyrosine

Abbreviations: THR, threonine; VAL, valine; ILE, isoleucine; LEU, leucine; TYR, tyrosine; PHE, phenylalanine; HIS, histidine; LYS, lysine; and TRP, tryptophan.

use of sulphur-based fertilizers to enhance the growth. As a result, it was hypothesized that during rotation, residual sulphur in the soil may be incorporated into the Desi chickpea and metabolized into sulphur amino acids. Previous studies demonstrated that the application of sulphur-containing fertilizer increased the content of sulphur amino acids, methionine and cysteine, in winter wheat grains as well as barley (Eriksen and Mortensen, 2002; Järvan et al., 2012). [b] Another probable reason may be associated with the biosynthesis of lysine, threonine, methionine and leucine from asparagine in plants (Galili et al., 2005). Within this pathway, there are two crucial enzymes involved with regulating methionine and threonine synthesis: cystathionine γ-synthase (CGS) which leads to the methionine synthesis and threonine synthase (TS) which catalyzes the threonine synthesis. Depending on the environmental stresses (e.g., drought), one CGS pathway tends to out-compete the other leading to differing levels of methionine. Shen et al. (1989) found that the concentration of methionine was higher in drought-stressed flatpea. Methionine levels in a hybrid Bermuda-grass were also found to increase from the control by 57% and 250% after 6 and 18 d of drought, respectively (Du et al., 2012). Taylor et al. (2008) also found that the deficiency of storage proteins in common bean lead to an increased synthesis of sulphur amino acid, especially cysteine, where content of sulphur amino acids (methionine + cysteine) raised above the FAO recommendation of 25 mg/g protein (equals to 2.5 g/ 100 g protein).

Based on the amino acid scores (Table 4.2.4), Desi chickpeas and hull-less barley flours (untreated or treated) were found to be limiting in threonine and lysine, respectively. Thus, the amino acid scores for the limiting amino acids ranged between 0.83 and 0.89 for Desi chickpea flour, and 0.61 and 0.69 for hull-less barley flour depending on the level of heat or tempering + heat (Table 4.2.4). *In vitro* protein digestibility (IVPD) for untreated and treated Desi chickpea and hull-less barley flour is given Table 4.2.4. The addition of heat and tempering increased the IVPD in barley samples from 72% to ~77% ($p<0.05$). In the case of Desi chickpea samples, there was no significant improvement and the IVPD of the chickpea samples were around 73 to 79% ($p<0.05$). In the case of chickpea, positive correlations were found between IVPD and lipid content ($r = 0.641$, $p<0.01$) and gelatinized starch content ($r = 0.535$, $p<0.05$), and negative correlations between IVPD and solubility ($r = -0.578$, $p<0.05$) and TIA ($r = -0.587$, $p<0.05$) (Table 4.2.5). For barley, IVPD was found to be positively correlated with tannin levels ($r = 0.759$, $p<0.01$) and negatively correlated with solubility ($r = 0.836$, $p<0.001$), total phenolics ($r = -0.769$, $p<0.001$), TIA ($r = -0.718$, $p<0.01$) and CIA ($r = 0.672$, $p<0.01$) (Table 4.2.5). Overall, findings suggest that

Table 4.2.4 Amino acid scores, limiting amino acid score, *in vitro* protein digestibility, and IV-PDCAAS values for untreated and treated flours prepared from Desi chickpeas and hull-less barley, with and without tempering and infrared heating to different surface temperatures, in reference to the FAO/WHO reported values (1991).

Treatment	THR	VAL	M+C[1]	ILE	LEU	P+T[2]	HIS	LYS	TRP	Limiting Amino Acid Score[3]	*In vitro* protein digestibility (%)[4]	*IV-PDCAAS*
a) Desi chickpeas												
Un-tempered; non-micronized	0.87*	1.18	1.01	1.49	1.15	1.36	1.69	1.23	0.95	0.85	76.46 ± 0.84^{ab}	0.65 ± 0.01^{b}
Un-tempered; heated to 115°C	0.87*	1.17	1.01	1.48	1.16	1.36	1.69	1.22	0.97	0.86	73.23 ± 2.89^{b}	0.63 ± 0.02^{b}
Un-tempered; heated to 135°C	0.88*	1.16	1.03	1.47	1.15	1.35	1.74	1.21	0.99	0.85	76.20 ± 0.79^{ab}	0.63 ± 0.01^{b}
Tempered to 20% moisture; heated to 115°C	0.85*	1.17	1.03	1.47	1.14	1.37	1.75	1.22	0.95	0.83	77.12 ± 2.03^{ab}	0.65 ± 0.01^{b}
Tempered to 20% moisture; heated to 135°C	0.89*	1.15	0.99	1.44	1.11	1.33	1.79	1.17	0.95	0.89	79.28 ± 2.84^{a}	0.71 ± 0.03^{a}
b) Hull-less barley												
Un-tempered; non-micronized	0.81	1.33	1.21	1.18	1.02	1.16	1.35	0.61*	1.08	0.61	72.30 ± 0.76^{B}	0.44 ± 0.00^{C}
Un-tempered; heated to 115°C	0.84	1.40	1.41	1.19	1.05	1.28	1.32	0.70*	1.52	0.69	75.47 ± 1.78^{AB}	0.52 ± 0.01^{AB}
Un-tempered; heated to 135°C	0.85	1.39	1.38	1.17	1.05	1.26	1.28	0.68*	1.53	0.66	78.59 ± 0.77^{A}	0.53 ± 0.01^{A}
Tempered to 20% moisture; heated to 115°C	0.87	1.42	1.32	1.18	1.04	1.27	1.31	0.68*	1.50	0.68	76.96 ± 1.51^{A}	0.51 ± 0.01^{B}
Tempered to 20% moisture; heated to 135°C	0.85	1.40	1.34	1.16	1.04	1.26	1.29	0.67*	1.48	0.68	76.92 ± 0.91^{A}	0.52 ± 0.01^{AB}

Notes:
[1]Methionine+Cysteine; [2]Phenylalanine + Tyrosine
[3]Measurements were made on one processing runs only.
[4]Measurements were made on each processing run and represented as the mean ± one standard deviation.
(*) Indicates the first limiting amino acid.
Abbreviations: THR, threonine; VAL, valine; ILE, isoleucine; LEU, leucine; TYR, tyrosine; PHE, phenylalanine; HIS, histidine; LYS, lysine; and TRP, tryptophan.

IVPD seems to improve as the levels of anti-nutritional factors decrease (especially, chymotrypsin/trypsin inhibitor activity and total phenolics), and as the solubility of the protein is reduced. The cause of the close digestibility results obtained from non-treated chickpea and barley flour could be due to differences in the composition of anti-nutritional factors. Barley flour was found to have a higher content of total phenolic and tannins which would act to cross-link proteins to reduce their digestibility, whereas chickpea flour showed a higher enzyme inhibitor activity, which may lead to a similar inhibition of protein digestibility. Another reason might be the limitation of the protocol. The equation: IVPD (%) = 65.66 + 18.10 × ΔpH_{10min} (eq. 3.19) set a starting IVPD results of 65.66, i.e., even with no change in pH, a 65.66% of IVPD could be obtained.

Table 4.2.5. Pearson correlation (pairwise) results describing the relationships between the physiochemical properties and *in vitro* protein digestibility of processed Desi chickpea and hull-less barley flours.

Source	Correlations
Chickpea	Lipid ($r = 0.641$, $p<0.01$), Sol ($r = -0.578$, $p<0.05$), TIA ($r = -0.587$, $p<0.05$), GS ($r = 0.535$, $p<0.05$)
Barley	Sol ($r = -0.836$, $p<0.001$), Tannins ($r = 0.759$, $p<0.01$), TPC ($r = -0.769$, $p<0.001$), TIA ($r = -0.718$, $p<0.01$), CIA ($r = -0.672$, $p<0.01$)

N = 15, df =13, Pearson correlation coefficients [$r = 0.514$ ($p<0.05$); $r = 0.641$ ($p<0.01$); and $r = 0.760$($p<0.001$)],
Abbreviations: Sol: solubility, TIA: trypsin inhibitor activity, GS: gelatinized starch content, TPC, total phenolic content

In vitro protein digestibility corrected amino acid score (IV-PDCAAS) for both untreated and treated flours are shown in Table 4.2.4. In the case of hull-less barley flour, all the IV-PDCAAS values of treated samples increased to ~0.52 compared to the un-tempered and non-micronized sample with the value of 0.44. However, the difference between different heat or moisture + heat levels was not significant ($p>0.05$). The low IV-PDCAAS of barley flour is due to the low levels of lysine present in the flour. In the case of Desi chickpeas, the significant increase in the IV-PDCAAS value was only found in the sample tempered to 20% moisture and heated at 135^{o}C, where the IV-PDCAAS increased from 0.65 to 0.71 ($p<0.05$). The high IV-PDCAAS of chickpea flour was not surprising considering its balanced amino acid profile.

Nosworthy et al. (2017b) determined the protein quality for a range of cooked pulses (soaked for 16 h, followed by boiling for 6-10 min depending on the pulse type), including red kidney beans, navy beans and whole green lentils. From the 2010 crop year, the authors reported that chickpeas were found to be limiting in tryptophan (AAS 0.61), had a true protein digestibility of 85% and had a PDCAAS score of 0.52. This differed significantly with the 2014 crop where tryptophan was not found to be limiting in the Desi chickpea, which resulted in much higher PDCAAS scores (0.65-0.71). In comparison, Nosworthy and House (2017) reported PDCAAS scores for red kidney beans (0.55), navy beans (0.67), whole green lentils (0.63), split red lentils (0.54), split green peas (0.50) and black beans (0.53), with all being limiting in the sulphur-containing amino acids (methionine and cysteine). In contrast, the authors reported PDCAAS scores for split yellow peas (0.64) and pinto beans (0.59), which were similar to chickpea, limiting in tryptophan. PDCAAS scores for rolled oats and whole wheat were reported to be 0.57 and 0.40, respectively (FAO/WHO, 1991).

PDCAAS scores are used for labelling purposes in the US regulatory system, to allow for the marketing of food products intended for people >1 year of age (i.e., non-infants) (Marinangeli et al., 2017). Based on the standard serving sizes of pulses (90 g) and cereals (110 g) (Corrected for the PDCAAS values; g proteins x PDCAAS), the amount of protein must be between 10% and 19.9% of the daily protein requirement (50 g) for non-infant foods to be labelled a '*Good source of protein'*, whereas at levels >20% can be labeled as a '*Excellent source of protein'*. Based on this criterion, the Desi chickpea flour in the present study could be

Table 4.2.6 Percentage of daily protein reference requirement, calculated protein efficiency ratio and protein rating for flours prepared from Desi chickpeas and hull-less barley, with and without tempering and infrared heating to different surface temperatures.

Treatment	Crude protein (CP) (g/100 g)	% Daily reference values – US. Regulatory system			Protein efficiency ratios & protein ratings – Canadian regulatory system			
		IV-PDCAAS	Corrected CP per serving (90 g pulses 110 g cereals)	% Daily reference value	Calculated $PER_{IV\text{-}PDCAAS}$	g/250 mL	CP (g/250 mL serving)	Protein rating (250 mL serving)
a) Desi chickpeas								
Un-tempered; non-micronized	25.4	0.65	14.9	29.7	1.62	97.2	24.7	40.1
Un-tempered; heated to 115ºC	25.1	0.63	14.2	28.5	1.58	97.2	24.4	38.4
Un-tempered; heated to 135ºC	26.2	0.63	14.8	29.7	1.57	97.2	25.5	40.1
Tempered to 20% moisture; heated to 115ºC	25.9	0.65	15.3	30.5	1.64	97.2	25.2	41.2
Tempered to 20% moisture; heated to 135ºC	26.6	0.71	17.0	34.0	1.77	97.2	25.9	45.9
b) Hull-less barley								
Un-tempered; non-micronized	11.4	0.44	5.6	11.1	1.11	156.4	17.8	19.8
Un-tempered; heated to 115ºC	10.7	0.52	6.2	12.3	1.31	156.4	16.7	21.9
Un-tempered; heated to 135ºC	10.0	0.53	5.9	11.7	1.33	156.4	15.6	20.8
Tempered to 20% moisture; heated to 115ºC	11.4	0.51	6.4	12.8	1.27	156.4	17.8	22.7
Tempered to 20% moisture; heated to 135ºC	11.2	0.52	6.4	12.8	1.30	156.4	17.5	22.8

labelled as an 'Excellent source of protein' since the % of the daily reference value is between 30-34% depending on the processing treatment (Table 4.2.6). In the case of barley flour used in the present study, it can be listed as a "Good source of protein', since the % of the daily reference value is between 11-13% depending on the processing treatment (Table 4.2.6).

In Canada, protein labelling is based on the use of the protein efficiency ratio (PER) and a protein rating methodology. Typically, PER values are determined using a rat bioassay which involves feeding the rats a known amount of test protein for 28 days and determination by dividing the amount of weight gained by the rat by the total amount of protein consumed. These PER values are then normalized to the PER of casein (2.5) to increase the consistency when comparing values (Marinangeli et al., 2017). Canadian Food Inspection Agency (CFIA) allows the use of PDCAAS values in its estimation using the formulae: PER = [$PDCAAS_{(sample)}$/$PDCAAAS_{(Casein)}$] x 2.5, using a PDCAAS score for casein of 1.00 (CFIA, 2017). This calculation is only used for estimation purposes, and is not permitted to extrapolate to foods (which would need to undergo proper validation – PER testing) (Marinangeli et al., 2017), nor does CFIA recognize the use of the *in vitro* PDCAAS over *in vivo*. However, Nosworthy and House (2017) obtained a strong correlation (R^2 = 0.9898) relating the IV-PDCAAS values to PDCAAS using a single protein source including casein, pea, faba bean and lentil protein isolates, also for processed (baked, extruded and cooked) red and green lentil flour with correlations with R^2 value of 0.9971 (Nosworthy et al., 2018). In the present study, calculated $PER_{IV\text{-}PDCAAS}$ values ranged between 1.62 to 1.77, and between 1.11 to 1.33 for Desi chickpea and barley flour, respectively (Table 4.2.6). PER values reported in the literature vary somewhat depending on how the seeds were processed. However, present values were reported within a similar range. For instance, Nosworthy et al. (2017b) reported for soaked/boiled Kabuli chickpeas to have a PER value of 2.32; Nosworthy et al. (2018) reported red lentils to range between 0.79 to 1.14 depending on the type of processing (extruded, cooked or baked); and Nosworthy et al. (2017a) found PER values for raw and extruded flour of 2.55 and 2.62.

The protein rating system uses those PER values combined with relative serving sizes for determination. In the current study, the Canadian Nutrient file (Government of Canada) was used to estimate the serving size for a 250 mL serving of flour, which is equivalent to 97.2 g and 156.4 g of chickpea and barley flour, respectively. This serving size is based on the Reasonable Daily Intake (RDI) value, which corresponds to the average serving of a food consumed under normal

food habits of Canadians (Marinangeli et al., 2017). The protein rating is determined by multiplying the crude protein content in the serving size by the PER value. If the Protein Rating ranges between 20.0-39.9 then the food is considered to be a '*Source of protein'*, whereas if >40.0 then it's considered an '*Excellent source of protein'*. Based on the present results, the Desi chickpea flour was determined to be an '*Excellent source of protein'*, whereas the barley flour could only be considered a '*Source of protein'* (Table 4.2.6).

4.2.5. **Protein quality of Desi chickpea-barley blends**

Based on the anti-nutritional data and protein quality of the flours alone, flours tempered to 20% moisture and heated to 135^{o}C were selected for blending experiments at different chickpea: barley ratios. The amino acid composition (g/100g flour), the concentration of essential amino acids (mg/g protein) and amino acid scores (based on the FAO reference pattern) for the chickpea: barley blended flours are given in Tables 4.2.7 and 4.2.8. For the barley flour alone, lysine was found to be limiting, whereas at ratios between 20:80 and 100:0 chickpea: barley, threonine was found limiting (Table 4.2.8). The *in vitro* protein digestibility data was found to be similar regardless of the blending ratio with a mean value of 78.6% ($p>0.05$) (Table 4.2.8). The addition of chickpea to barley flour, however, resulted in an increase from ~52% (100% barley flour) to ~59% IV-PDCAAS values at blending ratios of 20:80, 40:60 and 60:40 chickpea: barley ($p<0.05$), and then increased further to ~0.62 (80: 20 chickpea: barley; $p<0.05$), and then again to ~0.71 (100% chickpea flour) ($p<0.05$) (Table 4.2.8). Findings from this study indicate that the nutritional properties of barley can be enhanced with the addition of chickpea, however, the protein quality would be less than chickpea flour alone.

Table 4.2.7 Amino acid composition (g per 100 g of flour, as is basis) of Desi chickpea and barley blended flours. Flours were tempered to 20% moisture and heated at 135^{o}C. Samples include: (A) chickpea: barley = 0: 100; (B) chickpea: barley = 20: 80; (C) chickpea: barley = 40: 60; (D) chickpea: barley = 60: 40; (E) chickpea: barley = 80: 20; and (F) chickpea: barley = 100: 0. Measurements were made on one processing runs only.

	ASP	THR	SER	GLU	PRO	GLY	ALA	CYS	VAL	MET	ILE	LEU	TYR	PHE	HIS	LYS	ARG	TRP
A	0.75	0.32	0.57	2.73	1.25	0.48	0.49	0.22	0.54	0.15	0.36	0.76	0.33	0.55	0.27	0.43	0.67	0.18
B	1.08	0.38	0.83	3.19	1.48	0.58	0.55	0.22	0.62	0.16	0.51	1.02	0.43	0.75	0.55	0.66	0.97	0.17
C	1.57	0.48	1.05	3.57	1.40	0.68	0.69	0.26	0.72	0.19	0.63	1.23	0.47	0.94	0.66	0.93	1.31	0.20
D	1.97	0.55	1.23	3.89	1.38	0.77	0.79	0.26	0.81	0.21	0.76	1.46	0.57	1.10	0.78	1.18	1.67	0.22
E	2.41	0.64	1.47	4.32	1.33	0.88	0.92	0.30	0.93	0.25	0.90	1.68	0.66	1.31	0.91	1.47	2.11	0.23
F	3.20	0.78	1.64	4.74	1.09	1.02	1.04	0.33	1.04	0.31	1.04	1.90	0.72	1.44	0.88	1.76	2.67	0.27

Abbreviations: ASP, aspartate; THR, threonine; SER, serine; GLU, glutamate; PRO, proline; GLY, glycine; ALA, alanine; CYS, cysteine; VAL, valine; MET, methionine; ILE, isoleucine; LEU, leucine; TYR, tyrosine; PHE, phenylalanine; HIS, histidine; LYS, lysine; ARG, arginine; and TRP, tryptophan.

Table 4.2.8 Essential amino acid concentration (mg/g protein), amino acid scores, limiting amino acid score, *in vitro* protein digestibility, and IV-PDCAAS values of Desi chickpea and barley flours tempered to 20% moisture and heated at 135°C, blended at different ratios.

Chickpea: barley ratio	THR	VAL	M+C[1]	ILE	LEU	P+T[2]	HIS	LYS	TRP	Limiting Amino Acid Score[3]	*In vitro* protein digestibility (%)[4]	IV-PDCAAS
	Essential amino acid concentration (mg/g protein)											
0: 100	29	49	34	33	69	80	25	39	16			
20: 80	25	41	25	34	68	79	37	44	11			
40: 60	25	38	24	33	65	74	35	49	11			
60: 40	26	38	22	36	68	78	36	55	10			
80: 20	26	38	23	37	69	81	37	60	9			
100: 0	30	41	25	41	74	84	34	69	11			
1991 FAO/WHO reference pattern	34	35	25	28	66	63	19	58	11			
	Amino acid score											
0: 100	0.86	1.41	1.35	1.18	1.05	1.28	1.30	0.68*	1.50	0.68	76.92 ± 0.91^{A}	0.52 ± 0.01^{A}
20: 80	0.74*	1.18	1.01	1.21	1.03	1.25	1.93	0.76	1.03	0.74	78.27 ± 0.55^{A}	0.58 ± 0.00^{B}
40: 60	0.74*	1.09	0.95	1.19	0.98	1.18	1.83	0.85	0.96	0.74	78.87 ± 0.63^{A}	0.58 ± 0.00^{BC}
60: 40	0.76*	1.08	0.88	1.27	1.03	1.24	1.92	0.95	0.94	0.76	78.51 ± 1.61^{A}	0.60 ± 0.01^{BC}
80: 20	0.77*	1.09	0.90	1.32	1.04	1.28	1.96	1.04	0.86	0.77	80.02 ± 0.73^{A}	0.61 ± 0.01^{C}
100: 0	0.89*	1.16	1.00	1.45	1.12	1.34	1.81	1.18	0.96	0.89	79.28 ± 2.84^{A}	0.71 ± 0.03^{D}

[1]Methionine+Cysteine; [2]Phenylalanine + Tyrosine

[3]Measurements were made on one processing runs only.

[4]Measurements were made on each processing run and represented as the mean ± one standard deviation.

(*) Indicates the first limiting amino acid.

Abbreviations: THR, threonine; VAL, valine; ILE, isoleucine; LEU, leucine; TYR, tyrosine; PHE, phenylalanine; HIS, histidine; LYS, lysine; and TRP, tryptophan.

5. OVERALL CONCLUSIONS

Barley and Desi chickpeas represent important crops grown around the world especially in developing countries in sub-Sahara Africa, and are consumed as part of a complementary diet to give a complete set of essential amino acids to support human growth and development. Although they are both nutritionally valuable, they also contain various anti-nutritional properties that can adversely affect protein digestion and mineral absorption when consumed. Their flours also have a functional role in product applications based on their functional properties, such as solubility, foaming, emulsifying and water/oil binding abilities. As such, having knowledge of both the nutritional (i.e., protein quality) and functional properties is important in developing healthy products for consumption. Furthermore, what impact could processing have on protein quality and their flour functionality, alone or blended is important in order to innovate healthier ingredients. The present research investigated the use of tempering and infrared heating temperature on the protein quality and functional attributes of the resulting individual and blended flours.

In the first study, the impact of processing on the physicochemical and functional properties of individual and blended Desi chickpea and barley flours was examined. The composition of Desi chickpea flour was found to be ~25% protein, ~3% ash, 5% crude fat and 42% of total starch, whereas barley flour comprised of ~11% of protein, ~2% of ash, ~2% of crude fat and ~60% of total starch in barley flour, regardless of the processing conditions. Tempering and infrared heating were found to reduce the protein solubility in both flours, whereas the ability to bind oil was unaffected. Both flours also showed an increase in water binding abilities in response to tempering and infrared heating. The emulsifying and foaming properties differed, however. In the case of Desi chickpea flour, the emulsion activity increased, foam capacity decreased, and emulsion/foaming stability remained unchanged with tempering and heating. In contrast, hull-less barley flour showed a decrease in both emulsion activity and stability, and became non-foaming with tempering and infrared heating. The pasting properties of Desi chickpea flour were found to also be less sensitive to processing than the barley flour, in part due to the decreased level of the

gelatinized starch present. The physicochemical and functional properties relate to blended chickpea and barley flour showed a gradient change in accordance with the blending ratios.

The second study demonstrated that tempering and infrared heating are capable of increasing the protein quality of chickpea and barley flours without affecting overall protein content. Reducing anti-nutritive factors such as the phenolic and tannin content and the activity of protease inhibitors of these flours via processing were found to increase *in vitro* protein digestibility. This, in conjunction with an increase in the overall amino acid score, contributed to a higher *in vitro* PDCAAS value for processed barley and chickpea flours. Interestingly, while blending chickpea with barley was able to increase the overall protein quality of the barley flour, chickpeas alone had a higher *in vitro* PDCAAS score than any blend investigated in this study. This work highlights the functionality of *in vitro* methods for determining protein quality as well as indicating that the quality of the chickpea protein may be greater than previously suspected, at least with the seed examined from the 2014 Saskatchewan Harvest.

Based on this research, tempering both Desi chickpeas and hull-less barley to 20% moisture followed by infrared heating at 135^{o}C was sufficient conditions to both improve protein quality (IV-PDCAAS) and lower the levels of anti-nutritional compounds. In addition, blending barley flour with chickpea would act to improve the nutritional properties of the barley flour for consumption. However, for food aid products aimed at addressing acute malnutrition, a PDCAAS score > 0.70 is required according to the World Health Organization. As such, only the desi chickpea flour, tempered to 20% moisture and heated to 135^{o}C by infrared heating would qualify for inclusion in such products.

6. FUTURE STUDIES

The current research focused on the effect of tempering and infrared heating on the protein quality of Desi chickpea and barley flours and their blends. Results showed that processing conditions had an impact on both the functional attributes and protein quality within the flours, however, only 2 tempering and 2 surface temperatures were selected for examination. Additional work could include the addition of higher tempering levels and higher temperatures to induce a greater amount of protein denaturation and starch gelatinization without over-processing the seed during the heating process. This may have a more dramatic effect on both functionality and protein quality, however, results would be expected to follow similar trends as seen in the present study. Furthermore, comparing the processed chickpea and barley flours, in terms of their functionality and protein quality to other flours in the marketplace, such as wheat, soy, corn, rice, maize, pea, faba bean and lentil would be useful for putting the developed ingredients better in perspective. Although the functionality of the flours can be quantified, the relationship between functionality and product formulation is less clear. Further work, in the utilization of the processed flours and/or blends in various product formulations would be warranted to better tie ingredient functionality to the product performance.

In addition, the study could benefit from the development of standardized methods of measuring protein functionality, in particular, oil holding, emulsification and foaming. Without standardized methods, values reported in the literature tend to differ substantially between laboratories making it difficult to compare the results. For the development of standardized methods, one would first conduct a comprehensive survey of literature methods, develop a step-by-step procedure for a technician to follow, and then send out a selection of flour materials from different sources (labelled only with random codes) to multiple laboratories (6-10) for analysis. Methods would then be modified based on the feedback from independent laboratories. The final method would be repeatable (x3) for all independent labs. The standardized method would then be submitted for approval by an international body, such as the American Association of Cereal Chemists'.

In terms of protein quality, it would be important to correlate the *in vitro* protein digestibility work and IV-PDCAAS values with *in vivo* animal studies involving the rat bioassay for estimation of PER, true fecal digestibility, ilium fecal digestibility, PDCAAS and DIAAS. It is important to do *in vivo* animal studies due to the closer results they can get, however, the concern of scientific costs associated with animal studies, and ethical concerns surrounding the use of animals in experiments creates significant challenges. Further work on developing more reliable *in vitro* methods are thus of great importance for these reasons.

One of the most interesting findings arising from this work was the high protein quality of the Desi chickpea from the 2014 Saskatchewan Harvest. Further work in understanding the biosynthesis pathways associated with the conversion of asparagine in plants into lysine, threonine, methionine and leucine would be of significant scientific and economic interest. The ability to tailor this pathway by inducing environmental stress on the crop could lead to a chickpea ingredient very close to a complete protein source in terms of their essential amino acid. If this can be achieved, it would better compete with soy within the global protein ingredient market than other pulse proteins. However, a full economic evaluation would be needed to investigate the tradeoff between possibly a decreased crop yield versus a higher quality food ingredient. In addition to the biosynthetic pathway of the amino acids, a greater understanding of soil chemistry and agronomic practices should be considered, in relation to a potentially increased uptake of sulphur and its metabolism into thiol-containing amino acids.

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